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Ph. D. Dissertation

August 1970

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Space Sciences Laboratory

Grant No. 05 - 003 - 003

Lawrence Radiation Laboratory (UC Space Science Lab. and LRL Berkeley)

Berkeley, California

AEC Contract No. W-7405-eng-48

CHEMICAL STUDIES OF TERRESTRIAL AND EXTRATERRESTRIAL LIFE

Jerry Chih-Yuan Han

Ph.D. Thesis

August 1970

Chemical Studies of Terrestrial and Extraterrestrial Life

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Acknowledgement

I would like to express my gratitude to Professor Melvin Calvin for his encouragement and advice during the course of this work. This dissertation is in large part, due to his continuing enthusiasm and interest.

There are other members of the Department of Chemistry who are always willing and available to discuss any aspects of research. The Laboratory of Chemical Biodynamics deserves my thanks for many helpful suggestions and discussions I have had with many people. Mrs. Dell Roadman and Mr. William Newman are thanked for their generous and excellent technical assistance.

Many thanks are due to Jennie Gee, Evie Litton, and Marilyn Taylor for their considerable aid in preparing the manuscript.

I am also grateful to the National Aeronautics and Space Administration (Grant No. NSG 101-61) and the U.S. Atomic Energy Commission who have supported both myself and my research work.

Abstract

The problems of the time, manner and place of the beginning of life, both terrestrial and extraterrestrial, have become increasingly concerned by scientists for the past decade. This thesis have concerned itself with a study of the origin and evolutionary steps of life. Four aspects; Chemical Evolution, Biological Markers or Molecular Fossils, Chemical Taxonomy, and Possible Extraterrestrial Life are discussed.

The first portion of this thesis attempts to examine the historical record, trying to use modern analytical techniques to study from the present time backward into history. The second portion describes a comprehensive study of a new biological marker, 7- and 8-methylheptadecane. The structure of this branched C_{18} hydrocarbon has been confirmed by instrumental analysis as well as organic synthesis. Biosynthesis and geological significance have also been studied. The third portion of this thesis is concerned with the comparative biochemistry. The evolutionary step from microorganisms to higher plants and animals could be recognized by the variation in chemical compositions. The fourth portion of this thesis contains the experimental results of examination of extraterrestrial samples, and the possibility of extraterrestrial life is discussed.

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CHAPTER 1 INTRODUCTION

What is life? This question has been discussed by scientists for more than a century. If we view living and non-living matter, a line drawn between them would be arbitrary. Life in this view is associated with the complex metabolic apparatus of the cell enzyme, membranes, metabolic cycles, etc., and the point at which such a system becomes "alive" is undefinable. Most biologists, however, agree that living matter is uniquely defined by its genetic properties. According to this view, the feature that above all others distinguishes living matter is its mode of duplication. Living organisms select appropriate materials from their environment and transform them into accurate replicas of themselves. The self-replication of living systems extends to the occasional accidental variants which appear from time to time in populations. These mutants copy themselves when they replicate rather than copy the parental type from which they originated. Thus, for an organism to be called living, it must be capable of both replication and mutation; such an organism should evolve into higher forms. In order to study how life first started and how it evolved into higher forms, four aspects merit some consideration and discussion in this introductory chapter. They are: Chemical Evolution and the Origin of Life, Biological Markers or Molecular Fossils, Chemical Taxonomy, and Extraterrestrial Life.

1. Chemical Evolution and the Origin of Life

Life may be regarded as a step in the evolutionary history of our planet. Organisms survive due to a process that transmits information

from generation to generation. Non-living mixtures of chemicals do not currently give rise to life, but it is necessary to postulate that such a step must have occurred at some time in the past. The common taxonomic pattern of living organisms is in contrast with the extreme diversity of living organisms; this paradox is, however, in accord with the viewpoint that these organisms have evolved in numerous directions from a single origin.

Chemical evolution of biopolymers probably started after the earth had been formed from its primordial atmosphere. Probably the earth was condensed from a dust cloud 4.5-4.8 billion years ago.[1] As a result of chemical evolution, the first living organism was formed. The evidence indicates that the appearance of life on earth occurred at least 3200 million years ago. Barghoorn and Schopf[2-5] have reported the presence of blue-green algae fossils in a 3.2×10^9 year old sediment. Thus it leaves only about one and a half billion years for the period of chemical evolution.

There seems to be little doubt that the primitive atmosphere on earth was a reducing one in which hydrogen was the predominant element.[6,7] It is impossible to answer by direct observation the questions that arise about how our present atmosphere evolved. However, evidence available from a number of sources indicates that hydrogen gradually escaped from the earth and gave way to the oxygen atmosphere of today.[8] When the earth was forming, most of its carbon, nitrogen, oxygen and sulfur was probably in a thermodynamically stable form of methane, ammonia, water and hydrogen sulfide.[6-8] The carbon that made its appearance on the primitive earth was not in the oxidized state, e.g. carbon dioxide, but on the contrary it was in the reduced form, the simplest form of hydrocarbon, methane.[8]

The energy sources available for the synthesis of organic compounds under primitive earth conditions were ultraviolet light from the sun, electric discharges, radiation, heat and shock wave. Among these, probably sunlight was the principal source of energy. Solar radiation is emitted in the wave length range from 10^6 cm, radio wave to 10^{-14} cm gamma ray.[8] A great deal of this radiation, especially in the ultraviolet region is shielded today by the ozone layer in the upper regions of the atmosphere.

About one hundred years ago Charles Darwin first described the chemical evolution theory which could be initiated with an energy source and primitive chemical compounds. In a letter written on February 1, 1871 he stated:

> "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 acid 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." [8]

Oparin[9] and Haldane[10] presupposed a long period of chemical evolution before the appearance of life. The accumulation of organic matter on the primitive earth and the generation of duplicating molecules must have been two factors of prime importance in chemical evolution. The evolutionary process might be considered to have taken place in three stages: inorganic, organic and biological.

There have been many studies of the effects of ultraviolet light and electric discharges on various compounds.[11,12] The greatest amount of

work in the field of prebiological synthesis concerns the origin and formation of amino acids. This may be due to the fact that amino acids are readily formed under these conditions. Amino acids have been synthesized under simulated primitive earth conditions by the action of ultra-violet light, electric discharge, heat and ionizing radiation.[12] It is reported that 14 of the 20 amino acids found in proteins have been synthesized by abiogenic means.[8,11,12]. In one of the first experiments done, simulating the synthesis of organic matter on the primitive earth, Miller[13] exposed a mixture of methane, ammonia, water and hydrogen to an electric discharge from tesla coils for about a week, resulting in the formation of some organic compounds. Among these were the amino acids, glycine, alanine, aspartic acid and glutamic acid. More recently purines,[14] short-chain peptides,[15] and porphyrins[16] were also synthesized under simulated primitive-earth conditions. It is hoped that such experiments might lead ultimately to the structures and reactions which are essential to the formation of a living organism.

2. Biological Markers or Molecular Fossils

Another method of approaching the origin of life was also demonstrated by Darwin. In order to gain an understanding of the evolutionary history of the earth he studied the fossil record, in which morphologically recognizable entities could be placed in a chronological order which could trace this evolutionary path from earliest times. Organic geochemists are also examining a fossil record, not at the morphological level, but rather at the molecular level. The architecture of the molecular aggregates

which the living organism left in the rock can be recognized as characteristic of the organism itself. The characteristics of these molecular aggregates should be different from compounds synthesized abiologically. This method has been used to provide evidence in support of the proposed existence of life forms in Precambrian sediments older than three billion years.

As living organisms evolved, the hard parts, bones, shells, teeth, leaves etc. were left behind as fossils. These can be recognized from their morphology, their substance has decayed and changed to stable molecular forms. Nevertheless, these may be characteristic chemicals by which means evolution can be traced farther back in time beyond the limits imposed by the oldest known fossils. These fossils are on a molecular level and are thus called "molecular fossils".[17]

Some molecular fossils can be classed as biological markers if they possess all the following characteristics: a characteristic structural skeleton, reasonable stability to degradation over long periods of geological time, structural specificity derived from known biological sequences, and a low probability of formation by any non-biological means.

Treibs[18-20] carried out a series of experiments in the period of 1934-1945. He found some pigments in petroleum whose molecular characteristics could be related to present day animal and plant pigments. His findings introduced the chemical viewpoint of a biogenic origin for petroleum.

Another type of chemical substance can also be considered as a biological marker, if it existed as an optically active form. For instance,

amino acids may be synthesized in many abiological ways. However, an amino acid may be considered as a biological marker only when the L-form is predominant.

Various compounds of presumed biological origin have been isolated from petroleum[21-24], ancient sediments[25-28], and meteorites[29,30]. Among these classes of organic compounds the hydrocarbons have probably received the most attention. Meinschein[31] studied the solvent extracts of soil of environments varying from swampy regions to arid regions. They were found to contain more odd than even carbon numbered normal paraffins. Bray[32] reported that the ratio of odd over even carbon numbered n-paraffins significant in Recent sediments is approximately 2.4 to 5.5. However, the value decreases for older sediments, and it is close to 1.0 in petroleum.

The isoprenoid hydrocarbons with the regular head-tail-head-tail linkage have been sought in Precambrian sediments and petroleum.[24,33,34] These compounds can be derived from known biological precursors such as phytol, farnesol, bactoprenols $H[CH_2C(CH_3)=CHCH_2]_{11}OH$, solanesol $H[CH_2C(CH_3)=CHCH_2]_9OH$, dolichol $H[CH_2C(CH_3)=CHCH_2]_{20}OH$, etc.[35] While they have a certain probability of formation by non-biological means, they generally have been taken to be the residue of life forms.

3. Chemical Taxonomy

What was the impact of Darwin's evolutionary ideas on taxonomy? It is commonly stated, or implied, that these were revolutionary. Biologists talk of "affinities"; the Darwinian biologist substitutes "evolutionary

relationship". The pre-Darwinian might have drawn a diagram of the Scala Naturae; the Darwinian draws a "Family tree".

What Darwin attempted to show was that the evolutionary process "explained" the previously mysterious "natural groups". In other words, the possibility of making a generally agreed upon natural classification of organisms depends upon the process of evolution. This is emphatically not the same thing as saying that any and every natural classification must be phylogenetic. The relationship between the actual course of evolution and the patterns of organic variation, for which it is the aim of taxonomy to provide a map, is in itself a fascinating and complex study.

Natural classifications, constructed by the sum total of resemblances or differences in an indefinite number of characters, were made by taxonomists long before evolution was ever talked about. Such classifications can be made of any group of objects, whether living or not, which exhibit significant character correlations.

Chemical criteria can be used as confirmatory evidence to classify living organisms which were previously identified solely on the basis of morphological characteristics. Chemistry may have more to contribute than any morphological analysis, not only because of the relative evanescence of most plant tissues in geological deposits, but also because the biochemistry of evolutionary processes may be deduced from the presence of compounds from known diagenetic pathways. Chemical taxomomy, properly

applied, may be more useful than such subjective morphology. Many chemical compounds are so common that they have small taxonomic value, but others, notably the so-called secondary metabolites, are often restricted to certain taxa and may help to distinguish one group of plants, for example, from another. Often the variation in chemical composition can sharpen distinctions in determining taxa, and may also disclose hitherto unsuspected relationships. In recent years the development of new analytical techniques, such as gas chromatography, ultraviolet, infrared, nuclear magnetic resonance, and mass spectrometry, made these chemical taxonomy studies feasible. With the help of such methods, individual compounds from a complicated mixture can be identified unambiguously in a short period of time.

Eykman[36] reported in 1888 upon his work on alkaloids, and notes their frequency in certain families. A few years later von Romburgh[37] published quite extensive work on the occurrence in plants of acetone, methyl salicylate, and HCN. In a long series of papers published between 1917 and 1945, McNair[38-42] attempted to apply comparative chemistry generally to taxonomy. Fats and oils and their taxonomic significance were to prove of continuing interest to him, and in a paper[39] appearing in 1929 he considered more than 300 oils, fats, and waxes occurring in 83 families, in relation to climate and taxonomy. He divided the oil into drying, semi-drying, and non-drying, and noted other characteristics such as iodine numbers, saponification values, and specific gravities. In 1935 he turned his attention to alkaloids.[40,41] He remarked on the fact that each species of a genus such as Aconitum may have a different member of a group of closely-related

alkaloids; that any one alkaloid rarely occurs in more than one family but may occur in many members of one family.

It is becoming increasingly clear that living organisms have, chemically speaking, a rather uniform basic pattern. Within this basic uniformity, however, we may recognize relatively minor differences that are detectable by modern analytical techniques.

4. Extraterrestrial Life

Meteorites have always been objects of great interest to mankind. Today this interest is due less to the spectacular manner of their arrival on earth than to their bearing on the ancient question of the origin of the solar system. The development of science during the 19th and 20th centuries stimulated an increasingly intensive and comprehensive study of meteorites. It is not generally accepted that they consist of extraterrestrial material and that they originate from some source within the solar system itself. They must have formed very early in the history of the solar system, long before any geological structure in the crust of the earth.[43]

The meteorites can be classified according to their macroscopic appearance into iron, stony-iron, chondrites, and achondrites.[43] Metallurgical and mineralogical characteristics, together with differences in the fine structure of the chemical composition, give criteria for a sophisticated subclassification within each major group. Despite their wide variety of structures and compositions, meteorites and the returned lunar materials are the most primitive objects at present accessible for detailed study. They constitute a complex record of the early history of material in the solar system.

To study the possible occurrence of life forms or chemical evolutionary steps in extraterrestrial material, carbonaceous chondrites, a subgroup of chondritic meteorites have been more intensively studied now than at any time since 1806 when the first recorded carbonaceous meteorite fell in Alais, France[44]. On the basis of chemical criteria, the carbonaceous chondrites are divided into three distinct types.[45] All three contain nearly the same proportions of nonvolatile elements (Mg, Si, Fe, Al, etc.) but they differ markedly in their state of oxidation and content of volatiles (C, H, S, N, etc.) . Both of these increase in going from Type II to Type I. The origin of the carbonaceous chondrites is a subject of much controversy and no theory has yet been proposed which has gained universal acceptance.[43]

In 1961, Nagy et al.[46] reported that the mass spectrometric analysis of hydrocarbons extracted from the Orgueil, a Type I carbonaceous chondrite, indicated that the hydrocarbons could be of biogenic origin. This conclusion was based on the resemblances of hydrocarbons in the Orgueil meteorite to the hydrocarbons found in sediments and to those produced by living organisms. Anders[47] was especially critical of Nagy et al., offering ten specific criticisms which included pointing out the possibilities of contamination, abiotic synthesis, and also the fact that the data from the biogenic hydrocarbons did not agree with each other and thus could not be used as proper standards for evaluating the meteorite data. More recently, Nooner and Oro[48] reported that the hydrocarbons ranged from 0.1 to 25.8 ppm for Type I, 8.3 to 150 ppm for Type II, and 8.2 to 415 ppm for Type III carbonaceous chondrites. Isoprenoid hydrocarbons ranging from C_{18} to C_{20} were observed

in practically all of the meteorites. Besides hydrocarbons, many groups of organic compounds such as alcohols,[49] fatty acids,[50,51] phenolic compounds,[52,53] halogen-containing compounds,[49,54,55] amino acids,[52,53,56,57] bases,[56,58] and porphyrins[59] were also found in meteorites.

This highly controversial subject is, at its simplest level, a question of whether those compounds observed in the meteorites are biogenic or abiogenic in origin. Every compound found in meteorites, with the possible exception of isoprenoid hydrocarbons, has been synthesized in some type of abiogenic process. The n-alkanes may definitely be produced by Fischer-Tropsch synthesis[60] but at present this cannot definitely be said for isoprenoid hydrocarbons. The origin of those organic compounds is still a question. However, all those meteorites could be contaminated by terrestrial matter. For those samples which had a long previous exposure on the earth before they were analyzed, contamination could occur during impact, wrapping, transferring, and storage. Since all the analyses of meteorites are questionable, analysis of a recently fallen carbonaceous chondrite could solve part of the problem. This work will be discussed in part of Chapter 9.

The ambiguity heightens when scientists are searching for extraterrestrial life on earth by studying the immediately available sample-meteorites. The alternative way, perhaps a better way, to approach this problem is to search for extraterrestrial life on the Moon, Mars, and on other planets, or the return of samples to the earth for examination in our own laboratories. It is generally agreed that this investment is the most important scientific objective of the space program. This is necessary not only in order to

justify the high priority given bio-organic observations on the planet, but also equally important to serve as a guide for exobiology itself.

We would like to know whether life has originated elsewhere in the Universe, and we would like to prove whether an extraterrestrial form of life originated independently of life on the earth. We cannot assume if life is found somewhere else, that its origin was a separate event from that which gave rise to life on earth. It is conceivable that life arose only once in the solar system or in the whole Universe and that it was subsequently distributed throughout all the the planets. The one way we know of at the present time by which the answer to this question might be obtained is by comparing the chemical structure of the extra-terrestrial life with that of life on the earth.

It is now assumed by some astronomers that a large number of stars are accompanied by families of planets. Therefore, it is desirable to examine the variety of environments which these planets might be expected to provide, and compare this with the planetary environment which seems necessary to promote the development of life systems. Some planetary environmental factors for consideration are temperature, light, atmosphere and particle radiation.

The temperature range -30°C to 100°C is of the utmost significance to carbonaceous type life. The earth lies near the center of a so-called bio-thermal band surrounding the Sun, while Mars lies near its outer edge and Venus near its inner edge. This temperature range also determines the liquid water range which is of significance to the development of biological life.[61-65]

The primordial chemical environment of all planetary systems may be assumed to be similar. This assumption is based on the conclusion that planets are formed along with stars in the condensations in dust clouds. Probably the early gaseous environment of most planets consists of hydrogen, methane, ammonia and water. The size of these gaseous envelopes, which would be determined by the mass of the planet, would serve to screen the planet's surface from radiation.[66,67] The number of planets and satellites in our solar system leads us to conclude that other similar stars are likely to be accompanied at least by several planets. The distribution of the masses of our planets and satellites gives a basis for estimating the probability of a planet, of sufficient mass to hold an atmosphere, occurring in the biothermal band of a star similar to the sun. This probability would seem quite high considering our own solar system.

Clearly, either on earth or elsewhere at some time in the past, simple chemical molecules began to evolve gradually into complex self-reproducing molecules, or units, capable of undergoing biological evolution. Regardless of which planetary origin theory is accepted, it is generally assumed that a primordial planet might have contained only simple chemical molecules. If the life system has not formed in those planets it would be very desirable to look for the small molecules and to determine what is the chemical evolutionary stage on the planets.

CHAPTER 1 REFERENCES

1. J. Tyndall, "Fragments of Science for Unscientific People," D. Appleton and Co., New York, N.Y., 1871, p. 155.
2. J.W. Schopf and E.S. Barghoorn, Science 156, 508 (1967).
3. E.S. Barghoorn and J.W. Schopf, Science 152, 758 (1966).
4. E.S. Barghoorn, W.G. Meinschein, and J.W. Schopf, Science 148, 461 (1965).
5. E.S. Barghoorn and S.A. Tyler, Science 147, 3658 (1965).
6. H.C. Urey, "The Planets", University of Chicago Press, Chicago, Ill., 1952.
7. A.I. Oparin, "Origin of Life", English edition, Dover, New York, N.Y., 1953, p. 101.
8. M. Calvin, "Chemical Evolution," Clarendon Press, Oxford, England, 1969.
9. A.I. Oparin, Proiskhoxhderie Zhizni, Izd. Moskovskiy Rabochiy, Moscow (1924).
10. J.B.S. Haldane, Rationalist Annual, 148 (1928).
11. C.A. Ponnamperuma and N.W. Gabel, Space Life Sci. 1, 64 (1968).
12. R.M. Lemmon, Chem. Rev., 70 , 95(1970).
13. S.L. Miller, Science 117, 528 (1953).
14. J. Oró, Biochem. Biophys. Res. Commun. 2, 407 (1960).
15. S.W. Fox and K. Harada, Science 128, 1214 (1958).
16. G.W. Hodgson and C. Ponnamperuma, Proc. Natl. Acad. Sci. 59, 22 (1968).
17. G. Eglinton and M. Calvin, Sci. Amer. 216, 32 (1967).
18. A. Treibs, Annalen 517, (1935).

19. A. Treibs, Angew. Chem. 49, 682 (1936).

20. A. Treibs, Liebigs Ann., 510, 42 (1945).

21. W.G. Meinschein, Geochim. Cosmochim. Acta 22, 58 (1961).

22. G.T. Philippi, ibid. 29, 1021 (1965).

23. M. Blumer, Science 149, 722 (1965).

24. J.G. Bendoraitis, G.L. Brown and L.S. Hepner, Anal. Chem. 34, 49 (1962).

25. W.G. Meinschein, Bull. Am. Assoc. Petrol. Geologists 43, 925 (1959).

26. P A. Haug "Applications of Mass Spectrometry to Organic Geochemistry," Ph.D. Thesis, Univ. of California, Berkeley (1967).

27. G. Eglinton and M. Calvin, Sci. Amer. 216, 32 (1967).

28. R.B. Johns, T. Belsky, E.D. McCarthy, A.L. Burlingame, P. Haug, H.K. Schnoes, W. Richter and M. Calvin, Geochim. Cosmochim. Acta 30, 1191 (1966).

29. D.W. Nooner and J. Oró, ibid. 31, 1359 (1967).

30. J.M. Hayes, ibid. 31, 1395 (1967).

31. W.G. Meinschein and G.S. Kenny, Anal. Chem. 29, 1153 (1957).

32. E.E. Bray and E.D. Evans, Geochim. Cosmochim. Acta 22, 2 (1961).

33. J. Oró and D.W. Nooner, Nature 231, 1082 (1967).

34. J.J. Cummins and W.E. Robinson, J. Chem. Eng. Data 9, 304 (1964).

35. J. Han and M. Calvin, Ceochim. Cosmochim. Acta 33, 733 (1969).

36. J.F. Eykman, Ann. Jard. Bot. Buitenz. 7, 24 (1888).

37. P. Van Romburgh, ibid. 16, 1 (1899).

38. J.B. McNair, Bot.Gaz. 64, 330 (1917).

39. idem., Amer. J. Bot. 16, 832 (1929).

40. idem. Bull. Torrey Bot. Club 62, 219 (1935).

41. idem., ibid. 62, 515 (1935).

42. idem., Bot. Rev. 11, 1 (1945).

43. E. Anders, Space Sci. Rev. 3, 583 (1964).

44. L.A. Pages, and Dhombres-Firmas, J. Phys. Chem. d'Hist. Nat. 62, 440 (1806).

45. H.B. Wiik, Geochim. Cosmochim. Acta 9, 279 (1966).

46. B. Nagy, W.G. Meinschein and D.G. Hennessy, Ann. N.Y. Acad. Sci. 93, 25 (1961).

47. E. Anders, ibid. 93, 649 (1962).

48. D.W. Nooner and J. Oro, Geochim. Cosmochim. Acta 31, 1359 (1967).

49. M.H. Studier, R. Hayatsu and E. Anders, Science 149, 1455 (1965).

50. W.G. Meinschein, Space Sci. Rev. 2, 653 (1963).

51. B. Nagy and M.C. Bitz, Arch. Biochem. Biophys. 101, 240 (1963).

52. M.H. Briggs, Nature 191, 1137 (1961).

53. I.R. Kaplan, E.T. Degens and J.H. Reuter, Geochim. Cosmochim. Acta, 27, 805 (1963).

54. J.M. Hayes and K. Biemann, ibid. 32, 239 (1968).

55. A.L. Burlingame and H.K. Schnoes, Science 152, 104 (1966).

56. M. Calvin and S.K. Vaughn, Space Res. 1, 1171 (1960).

57. E.T. Degens and M. Bajor, Naturwiss. 49, 605 (1962).

58. R. Hayatsu, Science 146, 1291 (1964).

59. G.W. Hodgson and B.L. Baker, Nature 202, 125 (1964).

60. E. Gelpi, J. Han, D.W. Nooner, and J. Oro, Geochim. Cosmochim. Acta, in press (1970).

61. J.R. Antoniadi, Astron. Soc. Canada 27, 402 (1931).

62. W.M. Sinton and J. Strong, Astrophys. J. 131, 460 (1960).

63. G. de Vaucouleurs and D.H. Menzel, Nature 188, 28 (1960).

64. G. de Vaucouleurs,"Physics of the Planet Mars" Faber&Faber, London (1954).

65. J.A. Giordmaine, L.E. Alsop, C.H. Townes, and C.H. Mayer, Astron. J. 64, 332 (1959).

66. H. Urey, "Science in Space", Natl. Acad. Sci., Washington, D.C. (1960).

67. N.A. Weil, "Lunar and Planetrar Surface Conditions", Academic Press (1965).

CHAPTER 2 EXPERIMENTAL

The operations and processes described in this section are the general analytical experimental techniques used in this laboratory. The synthetic experiments and other techniques will be described in the other chapters. Since some portions of these procedures have been published elsewhere,[1-7] emphasis will be placed on modifications and innovations designed to meet the demands of contamination-free, ultra-micro organic compound identification and structure determination.

The first group of samples studied consisted of algae, photosynthetic and nonphotosynthetic bacteria, their constituents of hydrocarbon and fatty acid were determined. The second sample group consisting of petroleum, seep oils, rock samples and meteorites was examined according to the basic procedure reported by Eglinton et al.[8]

The analytical procedure used for living organisms is given in Figs. 1 and 2. Ten grams of dried cells, or organisms, were treated with 150 ml of 3:1 benzene and methanol for 15 min. with stirring and sonicated for 30 min. Then the total sample and supernatant were transferred to a Soxhlet apparatus and extracted for 8 hr. with the same mixture of solvents. The extraction solvents were evaporated to leave a dark colored residue.

The total extracted material was transferred to the top of an alumina column (100 g, 30 cm) previously washed with n-heptane (100 ml); it was then eluted with three solvents to give four fractions. The first fraction, containing the total aliphatic hydrocarbons, was eluted from the column with 100 ml of n-heptane (n-heptane fraction). The second and third fractions were obtained by elution with 100 ml and 150 ml of benzene, respectively. The second fraction (benzene fraction I) contained aromatic

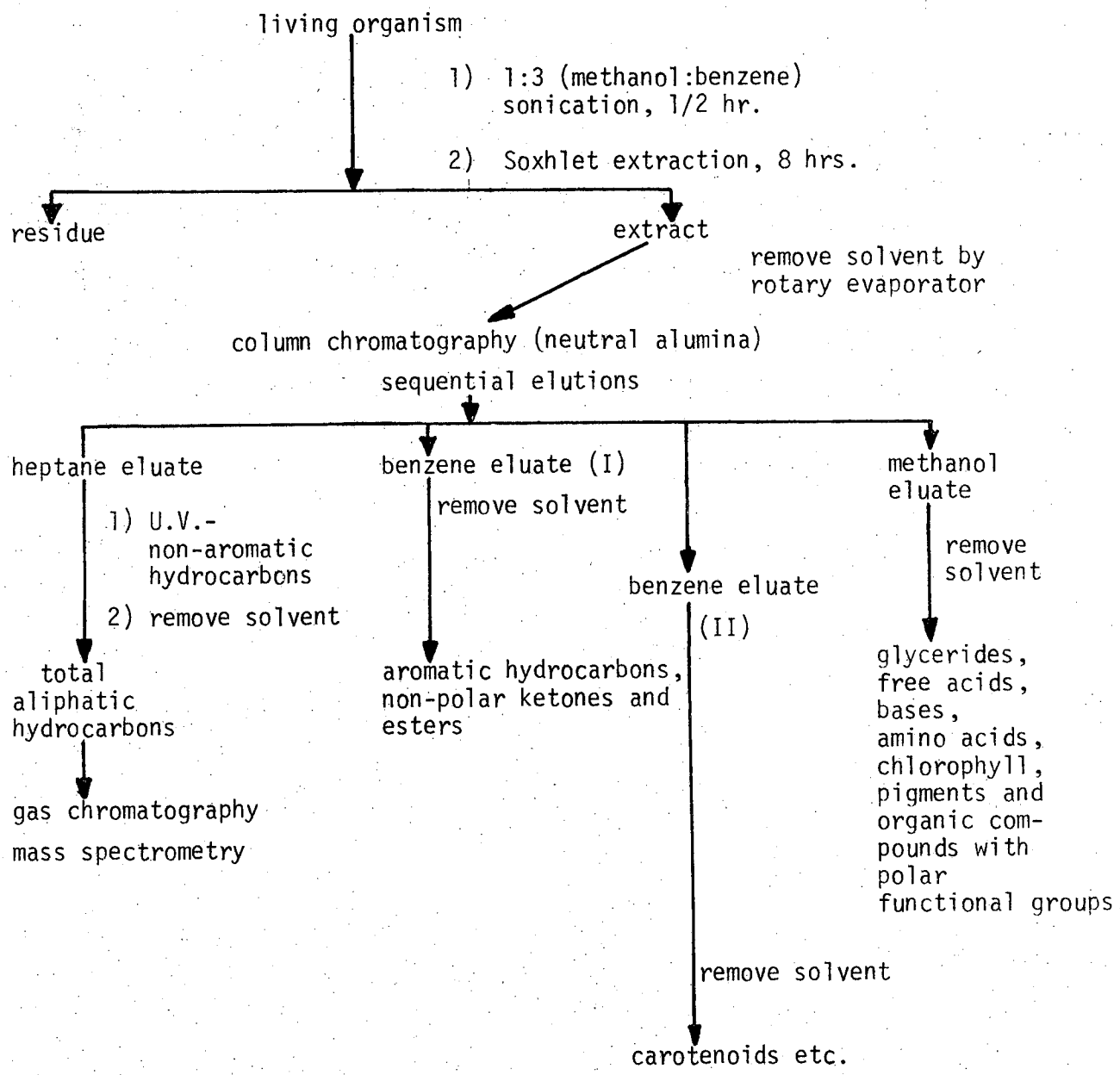

living organism
1) 1:3 (methanol:benzene) sonication, 1/2 hr.
2) Soxhlet extraction, 8 hrs.
residue
extract
remove solvent by rotary evaporator
column chromatography (neutral alumina)
sequential elutions
heptane eluate
1) U.V.-non-aromatic hydrocarbons
2) remove solvent
total aliphatic hydrocarbons
gas chromatography
mass spectrometry
benzene eluate (I)
remove solvent
aromatic hydrocarbons, non-polar ketones and esters
benzene eluate (II)
remove solvent
carotenoids etc.
methanol eluate
remove solvent
glycerides, free acids, bases, amino acids, chlorophyll, pigments and organic compounds with polar functional groups.

Fig. 1

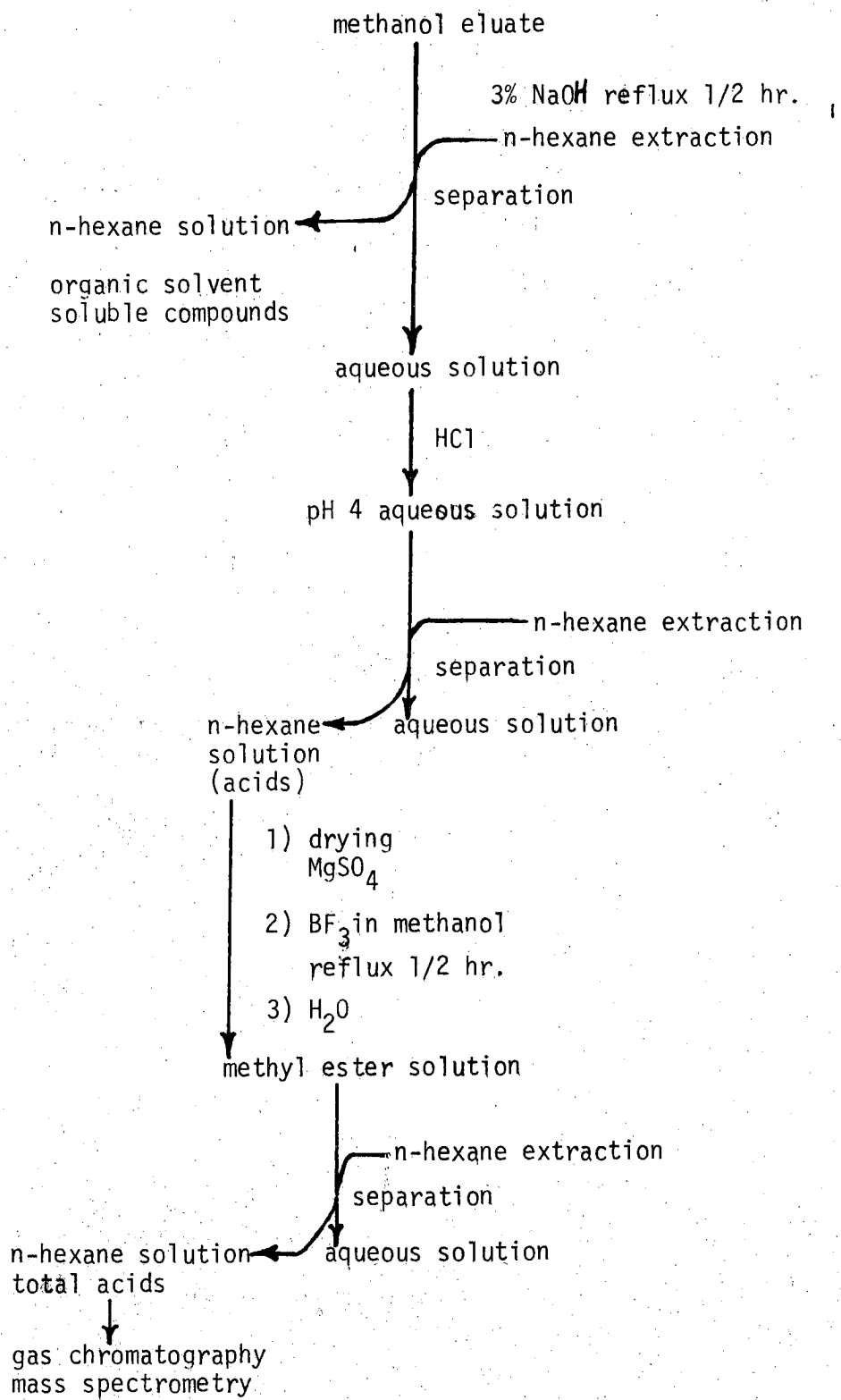

methanol eluate
3% NaOH reflux 1/2 hr.
n-hexane extraction
separation
n-hexane solution
organic solvent
soluble compounds
aqueous solution
HCl
pH 4 aqueous solution
n-hexane extraction
separation
n-hexane
solution
(acids)
aqueous solution
1) drying
MgSO4
2) BF3 in methanol
reflux 1/2 hr.
3) H2O
methyl ester solution
n-hexane extraction
separation
n-hexane solution
total acids
aqueous solution
gas chromatography
mass spectrometry

Fig. 2

and highly unsaturated hydrocarbons as well as nonpolar ketones.[9] Carotenoids are the major components in the third fraction (benzene fraction II). The fourth fraction, obtained by elution with 150 ml of methanol, contained glycerides, free acids, bases, amino acids, chlorophylls, and other organic compounds with polar functional groups.

The methanol fraction was used for fatty acid analyses. An aliquot of the methanol fraction was saponified with sodium hydroxide; impurities were removed by extracting with three 10 ml portions of n-hexane. Finally, the alkaline solution was acidified, and the fatty acids were extracted into three 10 ml portions of hexane. After drying with $MgSO_4$, the acids were methylated by refluxing the hexane solution with 1 ml of BF_3 in methanol. The hexane layer was removed, and the methanol layer was extracted with three 5 ml portions of hexane. The combined hexane extract was concentrated with a rotary evaporator. The samples were then analyzed by gas chromatography and mass spectrometry. All mass spectra were taken by a combination of an Aerograph 204 gas chromatograph and an A.E.I. MS-12 mass spectrometer.

The geological samples were treated according to the diagram in Fig. 3 & 4. Petroleum and seep oil samples were treated according to the same procedure except for the initial procedure which utilized column chromatography.

After the outside surface of the rock was removed by a clean water-cooled diamond saw, the larger rock segment was wrapped in aluminum foil and fragmented using a hammer. The chips were collected and washed in a flask containing a mixture of benzene and methanol. A Mettler Electronics Ultrasonic Vibrator (ml. 5 - over 1000 peak watts at 28Kc) was used for

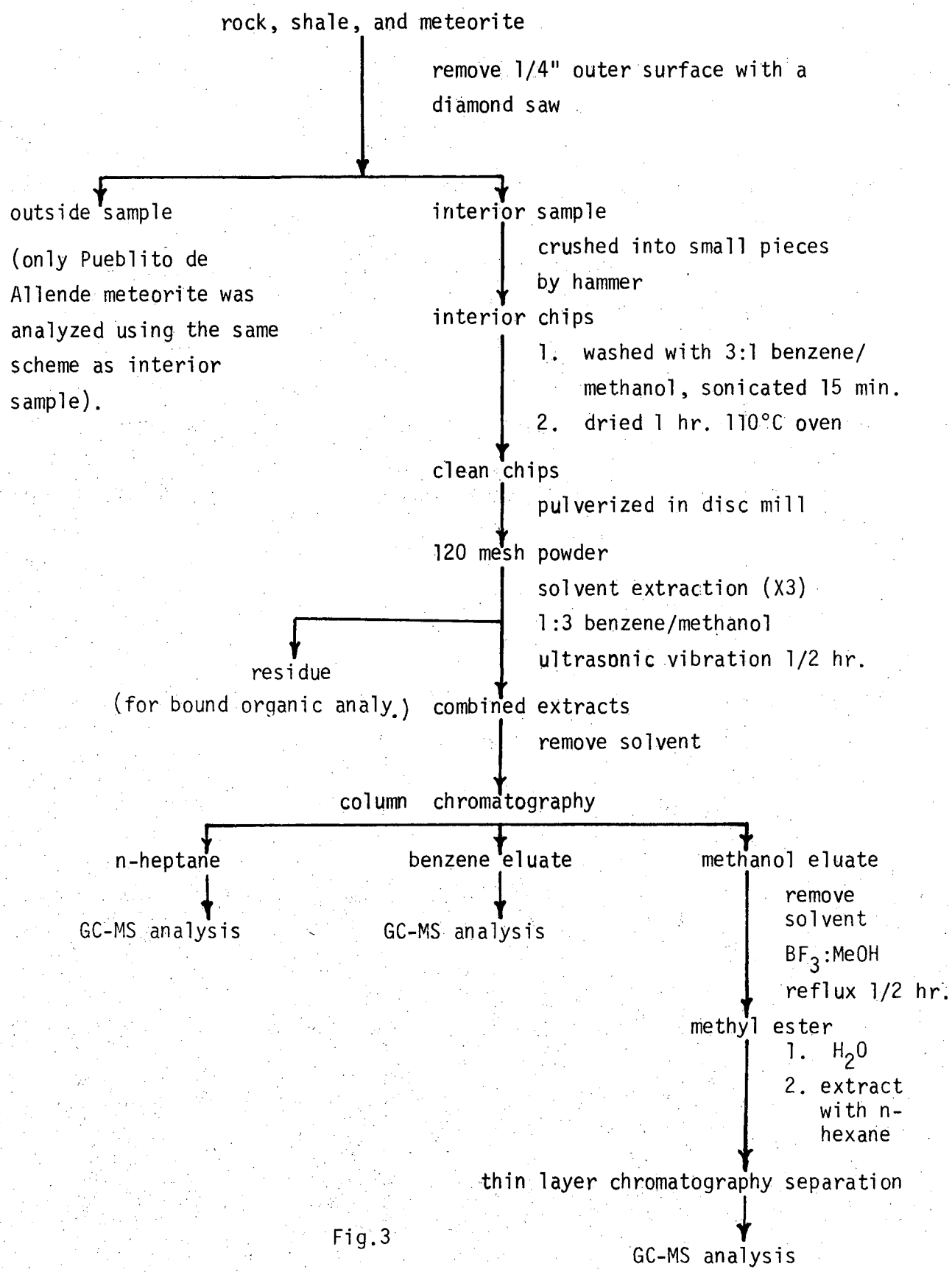

rock, shale, and meteorite
remove 1/4" outer surface with a diamond saw
outside sample
(only Pueblito de Allende meteorite was analyzed using the same scheme as interior sample).
interior sample
crushed into small pieces by hammer
interior chips
1. washed with 3:1 benzene/methanol, sonicated 15 min.
2. dried 1 hr. 110°C oven
clean chips
pulverized in disc mill
120 mesh powder
solvent extraction (X3)
1:3 benzene/methanol
ultrasonic vibration 1/2 hr.
residue
(for bound organic analy.)
combined extracts
remove solvent
column chromatography
n-heptane
benzene eluate
methanol eluate
GC-MS analysis
GC-MS analysis
remove solvent
BF3:MeOH
reflux 1/2 hr.
methyl ester
1. H2O
2. extract with n-hexane
thin layer chromatography separation
GC-MS analysis

Fig.3

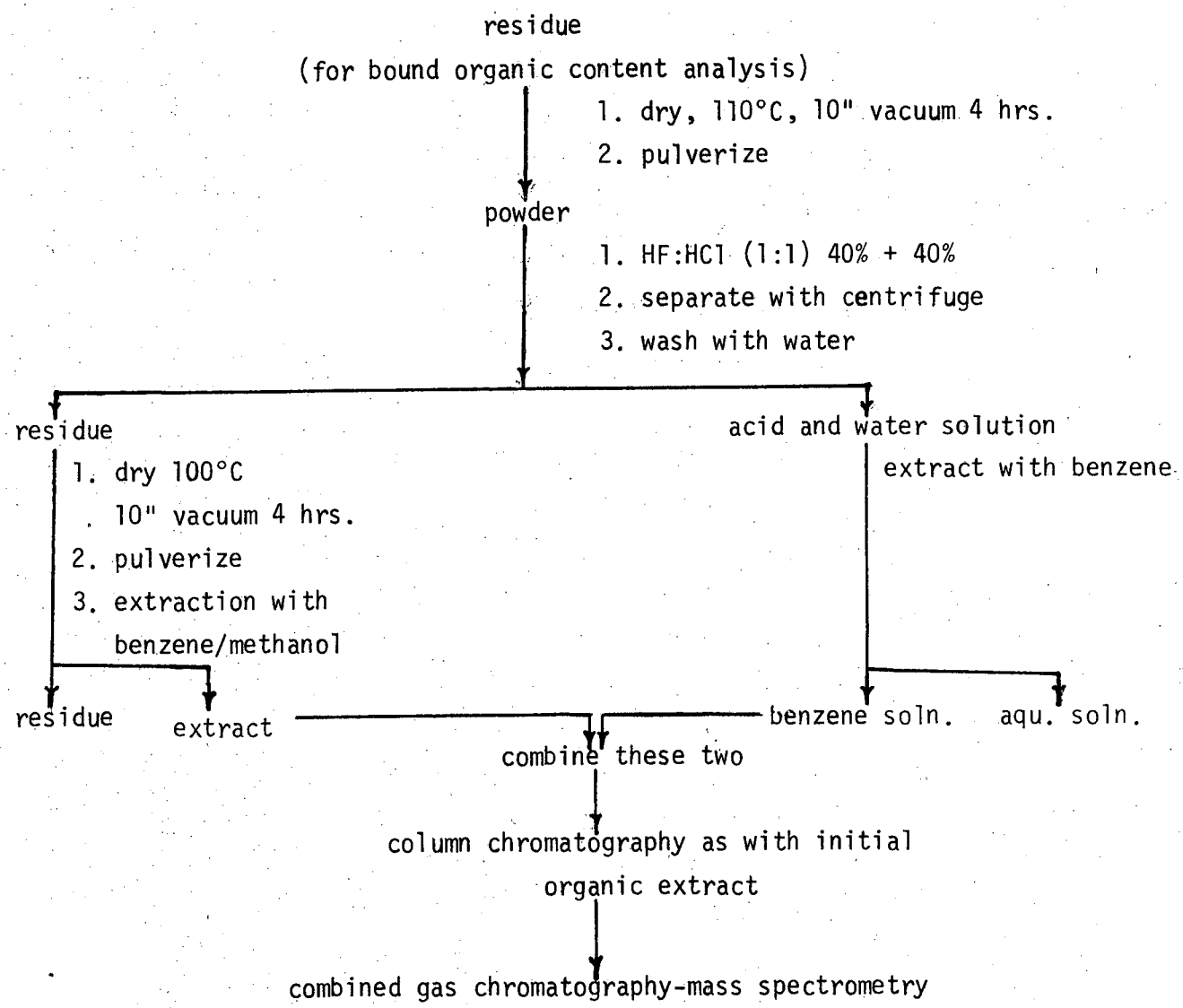

residue
(for bound organic content analysis)
1. dry, 110°C, 10" vacuum 4 hrs.
2. pulverize
powder
1. HF:HCl (1:1) 40% + 40%
2. separate with centrifuge
3. wash with water
residue
1. dry 100°C
10" vacuum 4 hrs.
2. pulverize
3. extraction with
benzene/methanol
acid and water solution
extract with benzene
residue
extract
benzene soln.
aqu. soln.
combine these two
column chromatography as with initial
organic extract
combined gas chromatography-mass spectrometry

Fig. 4

the washing process. The chips were dried in an oven and pulverized by a disc mill (Model 8701, type T250 Laboratory Disc Mill; Angstrom, Inc., Chicago, Ill.). The pulverized powder was less than 120 mesh in size. The powder was extracted three times with 3:1 benzene/methanol using an ultrasonic vibrator. After centrifugation, the supernatant solution was removed, yielding the "total extract". The total extract was fractionated by column chromatography and analyzed by gas chromatography and mass spectrometry.

Distillation was used to purify HCl; however, hydrofluoric acid could not be distilled in a glass apparatus. Kwestroo and Visser's [10] method was modified in this laboratory to purify HF. A closed Teflon box containing two Teflon beakers was made. One beaker contained crude HF and one contained distilled water. After 4-5 days, the concentration equilibrium could be reached.

Freshly prepared hydrogen fluoride and HCl was added drop by drop to the rock residue and stirred with a Teflon-coated magnetic rod. The digestion was considered complete when no further SiF_4 bubbles escaped from the solution. Separation and washing followed the digestion process. After the residue was dried at 110°C under 10" H_2O vacuum, it was extracted with 3:1 benzene/methanol, while the HF solution was extracted with benzene alone. Two extracts were then combined, yielding the "total bound organic content". After the solvent was removed the residue was fractionated by column chromatography and analyzed as for the initial free organic extract.

1. Problem of Contamination

During the last decade scientists from all over the world have employed the newest and most sensitive analytical techniques, such as ultraviolet, nuclear magnetic resonance, infrared, mass spectrometry and gas chromatography and electron spin renonance spectroscopy, to investigate the chemical composition and molecular structure of organic material from a wide variety

of natural origins. Contamination becomes a serious problem when research is directed to extraterrestrial material which usually contains only a micro-quantity of organic compounds. The question to be answered thus is whether or not these meteorites contain traces of life, proving the existence of extraterrestrial biological activity, or whether such staces are merely contamination by terrestrial matter. The samples may be contaminated not only in the laboratory, but also at the site of impact, by atmospheric penetration, by wrapping, transferring and storage.

During the laboratory handling and analytical processes, contamination may also be introduced into a sample. Solvents, chromatographic materials and equipment are the most obvious sources of contamination during analysis. In this laboratory a purified reagent grade of solvent was purchased and the purification was carried out before the solvent was used. The solvents were distilled by using a glass comumn of 40 theoretical plates with a reflux ratio of 6:1. 250 ml of the distilled solvent was concentrated to 1 ml and then analysed by gas chromatography. Only those solvents containing less than 1 microgram of detectable impurities in 250 ml were suitable for this work. The column chromatographic materials, silica gel (Baker Analyzed Reagent) and alumina (Alupharm Chemicals) were baked at 130°C for 24 hours and solvent-washed before use to eliminate contamination. To avoid contamination by equipment, all glassware was cleaned ultrasonically first with organic solvent and then with distilled water. All critical analyses were carried out in a clean cabinet with a continuous stream of filtered air.

A possible way to establish the indigenous nature of the biological compounds in extraterrestrial material is to determine whether the organic constituents differ from those of similar terrestrial materials. Homogeneous distribution of organic compounds in a sample stone from outside to inside and optical activity are the two commonly used methods of proving

certain organic materials indigenous to extraterrestrial material.

2. Extraction.

Two types of extraction were used in the experimental procedure:

a) Soxhlet-type extraction--an all-glass extractor was heated to moderate reflux temperatures by means of an electric heating mantle, with a power-stat controlled output. A Teflon covered magnetic stirrer was placed in the flask to prevent bumping. Eight hours of extraction time was usually used.

b) Ultrasonic-type extraction--a Mettler Electronic Ultrasonic Vibrator (M 1.5--over 1000 peak Watts at 28 Kc) was used for the extraction. The sample and solvent were placed in a covered flask or centrifuge tube which was placed halfway into the ultrasonicator water. Solvent to sample ratio was greater than 20:1 to prevent precipitation during sonication. Extractions were carried out at three half-hour intervals, using fresh solvent every time.

These two methods gave approximately equal efficiency for pulverized rock and sediment. With dried organisms, the ultrasonic extraction yielded more extracted residue than Soxhlet extraction. Therefore, a combination of the two methods was used for the microorganism analyses.

3. Chromatography

The history of chemistry is characterized by the intensive investigation of increasingly specialized areas in the various chemical technologies. Such efforts have resulted in the development of a number of useful analytical tools for the study and investigation of complex mixtures, such as absorption and partition chromatography. This technique was invented by Dr. A.J.P. Martin[11] who is also responsible for liquid-liquid partition chromatography

and advances in paper chromatography. These powerful techniques were originally applied to the investigation of complex biochemical mixtures. The potentialities of the method became apparent to organic chemists almost immediately and intensive research on chromatography was carried out in laboratories throughout the world. In recent years the development of new techniques such as column, thin layer and gas chromatography has made the separation of complicated organic mixtures feasible. And the mass spectrometry which has served as a structural detector of gas chromatography, has made the study of micro-quantity organic mixtures possible. Analytical methods used for this work are discussed individually as follows:

A. Column Chromatography

Separation of organic compounds by their differences in polarity by column chromatography is a well established procedure and a variety of adsorbents and solvents have been used for the purpose. The column was pre-washed with n-heptane before chromatography. Absorbent to sample ratios were generally higher than 50:1 and the column diameter was in the range of 1 cm to 3 cm. The chromatographic column was developed with n-heptane, benzene and methanol consecutively.

B. Thin-Layer Chromatography

In thin-layer chromatography an absorbent is applied to a supporting plate, usually glass, in a thin layer. Generally, a binding agent is used to adhere the adsorbent to the supporting glass. The mixture of adsorbent and binder are applied as a thin slurry and the excess moisture is removed under varying temperature and time depending on the desired degree of activity. The desired activity and developing solvent are the main controllers of good separation of organic compounds with different functional groups.

Silver-nitrate TLC[12] is used to separate saturated compounds from unsaturated ones. Five to ten percent of $AgNO_3$ is added to silicagel G (Applied Science Lab, Inc.), and a slurry made with distilled water before use. The plate is pre-developed with ethyl acetate and activated in the oven. The final development of the plate is with n-hexane/ether while saturated and unsaturated methyl esters are separated.

C. Gas Chromatography

In 1952 James and Martin[13] published their classic paper describing both the gas-liquid partition technique and a relatively simple apparatus for resolving aliphatic carboxylic acids. Since then, a new literature has developed which is devoted to this technique and now includes more than 20,000 publications. The determination of the composition of the effluent gas stream can be carried out conveniently, and with high sensitivity, by any of a number of relatively simple detectors. Even with the simplest form of thermal conductivity cell, for example, it is possible to determine parts per million concentrations of impurities. This detection limit can be extended to the parts per billion level with the more elegant flame ionization detector.

In 1956 at the first symposium on gas chromatography held in London, Martin[14] predicted: "We should be able to work from the milligramme down to the microgramme scale. Of course, that will imply that we decrease the diameter of our column correspondingly. We shall have columns only two-tenths of a millimeter in diameter, and these will carry, I believe, advantages of their own...."

It was not long before this prediction was fulfilled. Golay[15] first reported the use of capillary columns coated on the inside walls with liquid phase. The high resolution capillary column leads the way to

separate and to identify the complicated mixtures in microquantities.

Stainless steel, although relatively expensive, is often used in capillary columns, since it is stronger and more durable than glass and provides a nonpolar surface with excellent heat transfer properties. The stainless steel tubing 0.006, 0.010, and 0.020 inches in diameter were purchased from Handy and Harman Co., Norristown, Pa. The actual coating of the columns was accomplished in this laboratory. The capillary columns exhibited efficiencies between 150 to 500 theoretical plates per foot for 0.01 in. I.D. column and 150 to 350 theoretical plates per foot for 0.02 in. I.D. column. The columns made and used for this work were as follows:

a. stainless steel tubing, 100 ft., 0.01 in. inside diameter coated coated with Apiezon L (hydrocarbon grease);
b. stainless steel tubing, 100 ft., 0.01 in. inside diameter, coated with Polysev (7-ring polyphenylether);
c. stainless steel tubing, 100 ft., 0.01 in. inside diameter, coated with OV-17 (phenyl-methylsilicone, 50% phenyl);
d. stainless steel tubing, 100 ft., 0.01 in. inside diameter, coated with HI-EFF-4BP (Butane-1,4-diol Succinate);
e. stainless steel tubing, 200 ft., 0.01 in. inside diameter, coated with 50% of Igepal CO-880 [nonylphenoxypoly(ethyleneoxy) ethanol] and 50% of silicone GE SF-96 (silicone rubber);
f. stainless steel tubing, 100 ft., 0.01 in. inside diameter, coated with OV-1 (Dimethyl silicone);
g. stainless steel tubing, 150 ft., 0.01 in. inside diameter, coated with GE SR-96 (silicone rubber);
h. stainless steel tubing, 750 ft., 0.02 in. inside diameter, coated with 50% of Igepal CO-880 [nonylphenoxypoly(ethyleneoxy)ethanol]
i. stainless steel, 25 ft., 0.25 in. inside diameter, containing 3% SE-30 and 60 to 80 mesh Gas-Chrom Q;
j. stainless steel, 25 ft., 0.125 in. inside diameter containing 3% OV-1 and 60 to 80 mesh Gas-Chrom Q;

The instruments used for this analytical work are listed as follows:

a. An Aerograph A90 P-2 equipped with a thermal conductivity detector was used for all preparative work. Helium was used as the carrier gas; flow rate was 35-55 ml/min.
b. An Aerograph 204 with a modified injection system was used for capillary analytical work. It had a hydrogen-flame ionization detector with helium as the carrier gas; flow rate was approximately 2.5 ml/min. for 0.01 in. inside diameter capillary column.
c. A Perkin-Elmer 900 equipped with a flame ionization detector was used for this work.
d. A Barber-Colman 5000 equipped with a flame ionization detector was used.

4. Combined Gas Chromatography-Mass Spectrometry.

Mass spectrometry, long used in the petroleum industry and more recently in the chemical industry, is one of the finest single tools available for tha analysis of volatile chemical compounds. It is a rapid, precise method, uses sample sizes on the order of a few micrograms, and is often capable of identifying single components even if previously obtained standard mass spectra are not available. for comparison purposes.

The combination of a gas chromatograph with a mass spectrometer has proved to be an extremely useful tool for the rapid and positive identification of chromatographic fractions. Gas chromatographic data alone are often ambiguous, and the identification of sample components by this means can be obtained with assurance only by the expenditure of considerable time and effort. Usually the samples are collected in a cold trap. This system is time consuming and suffers from many disadvantages, particularly those which are associated with the collection, handling, and introduction of the sample into the mass spectrometer.

Ryhage[16,17] and Biemann[18] described combination gas chromatography-mass spectrometers which employed molecular separators coupled in series between the column and the inlet line of the mass spectrometer. With this technique, the sample-to-carrier gas ratio was increased at least 100 times, and good mass spectra were obtained from less than 1 microgram of material. Nevertheless, these molecular separators contributed too much sample loss when the carrier gas flow rate was only a few millimeters per minute.

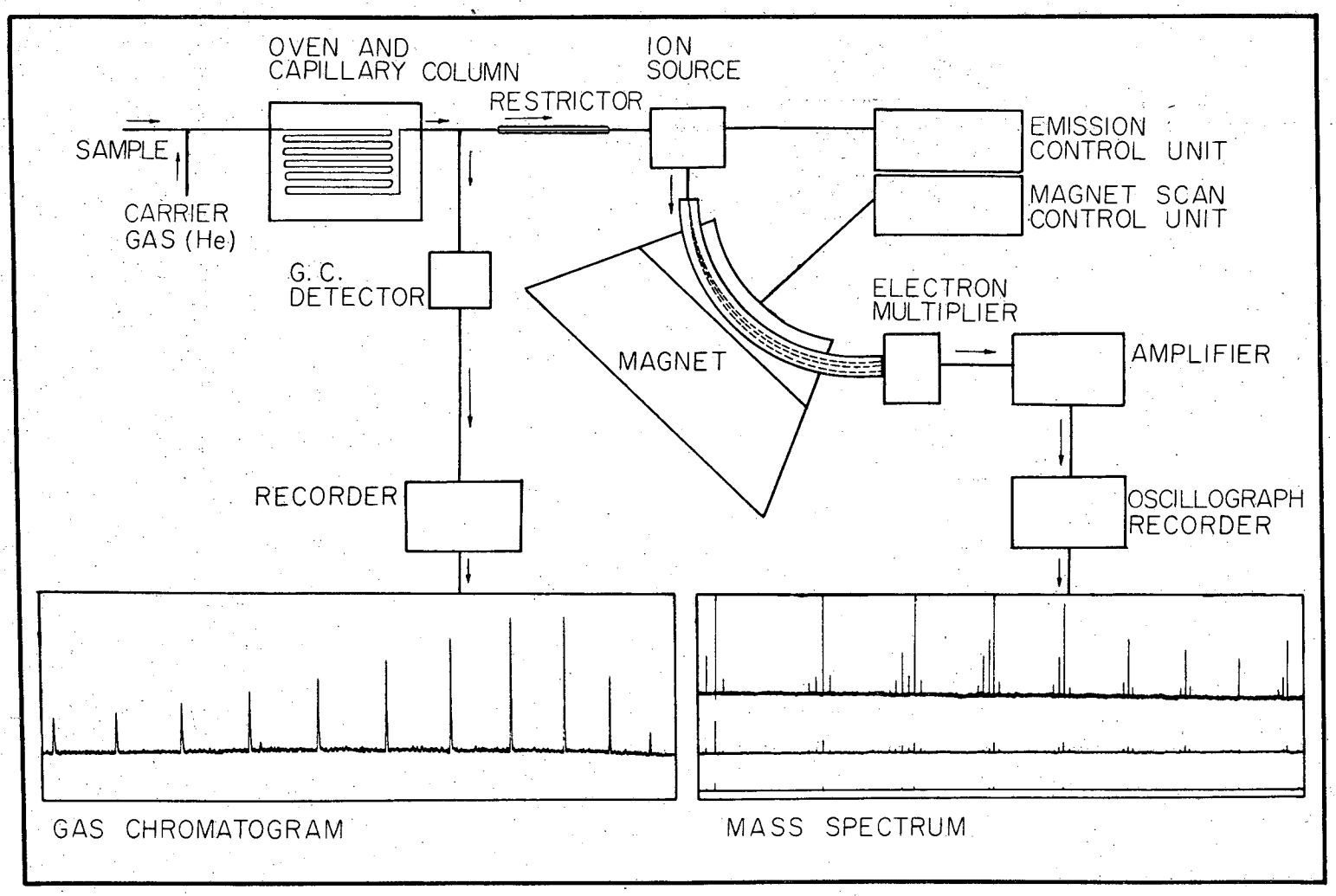
The system developed in this group (Fig. 5) connecting a T-joint to the outlet of the column and the GC effluent split into two fractions. Approximately 50% of the effluent went to the flame ionization detector and another 50% went through a 0.002 in. I.D. capillary restrictor before it went into the mass spectrometer. So far as is known at the present time, this method is the best one working for low carrier gas flow rate, high resolution capillary columns. However, its use is restricted to capillary columns, because the effluent goes directly into the ion source without any enrichment.

An AEI-MS-12 mass spectrometer was used for this system. Spectra were determined at an ionizing voltage of 70 eV and an ionizing current of 70 uA. The temperature of the ion source was generally set at 200°C. Each spectrum was scanned in three seconds. The spectra were recorded on an oscillograph recorder.

5. Other Physical Mesaurements

   a. IR spectra--infrared spectra were recorded on a Perkin-Elmer 257 instrument using a thin film from the liquid. Good IR spectra were obtained by using a micro-beam condenser and sodium chloride micro plates.

Figure 5: Block Diagram of the Gas Chromatograph - Mass Spectrometer combination.


OVEN AND
CAPILLARY COLUMN
ION
SOURCE
RESTRICTOR
SAMPLE
CARRIER
GAS (He)
EMISSION
CONTROL UNIT
MAGNET SCAN
CONTROL UNIT
G. C.
DETECTOR
ELECTRON
MULTIPLIER
MAGNET
AMPLIFIER
RECORDER
OSCILLOGRAPH
RECORDER
GAS CHROMATOGRAM
MASS SPECTRUM

XBL 693-4157

b. UV spectra - all spectra were measured on a Cary-14 spectrometer.

c. NMR spectra - all NMR spectra were recorded on a Varian A-60 spectrometer, at 60 Mc, with CCl_4 as solvent and tetramethyl silane as internal standard. A 50 μg sample was measured on the Varian spectrometer, at 100 Mc. Multiphases were used and the signals were recorded and averaged by a computer.

d. Polarimeter - optical activity was measured on an ETL-NPL-Automatic Polarimeter Type 143A, with sodium D line, 10 ohl load, and 90 mv scale.

e. Radioactivity - radioactivities were determined by a Tri-Carb liquid scintillation spectrometer. Toluene scintillation solution was used for all carbon 14 and tritium labeled compounds. The scintillation was prepared in this laboratory. 4.5 gr. of PPO and 1.0 gr. of dimethyl POPOP were transferred to 1 liter of toluene to make the scintillation concentration solution. Toluene was added to 50 ml of scintillation solution and 62.5 ml of colosolve #3 in order to make 1 liter of final solution.

CHAPTER 2 REFERENCES

1. G. Eglinton, P.M. Scott, T. Belsky, A.L. Burlingame, W. Richter and M. Calvin, in "Advances in Organic Geochemistry" G.D. Hobson and M.C. Louis (Eds.), Pergamon Press, London, 1966, pp. 41-74.

2. W. Van Hoeven, J.R. Maxwell, and M. Calvin, Geochim. Cosmochim. Acta 33, 877 (1969).

3. J. Han, E.D. McCarthy, M. Calvin and M.H. Benn, J. Chem. Soc. (C) 2985 (1968).

4. J. Han and M. Calvin, Geochim. Cosmochim. Acta 33, 733 (1969).

5. T. Belsky "Chemical Evolution and Organic Geochemistry" Ph.D. Thesis, University of California, Berkeley, California 1966.

6. E.D. McCarthy, "A Treatise in Organic Geochemistry", Ph.D. Thesis, University of California, Berkeley 1967.

7. W. Van Hoeven, "Organic Geochemistry", Ph.D. Thesis, University of California, Berkeley, 1969.

8. G. Eglinton, A.G. Douglas, J.R. Maxwell, J.N. Ramsay and S. Stallberg-Stenhagen, Science 153, 1133 (1966).

9. W.G. Meinschein and G.S. Kenny, Anal. Chem. 29, 1153 (1957).

10. W. Kwestroo and J. Visser, Analyst 90, 297 (1965).

11. A.J.P. Martin and R.L.M. Synge, Biochem. J. 35, 1358 (1941).

12. L.J. Morris, Chem. and Ind. (London) 1238 (1962).

13. A.T. James and A.J.P. Martin, Biochem. J. 50, 679 (1952).

14. A.J. Davies and J.K. Johnson in "Vapour Phase Chromatography", D.H. Destry (Ed.) Academic Press, New York, 1957, p. 185.

15. M.J.E. Golay in "Gas Chromatography", V.J. Coats *et al*. (Eds.), Academic Press, New York, 1958, p. 1.

16. R. Ryhage, Anal. Chem. 36, 759 (1964).

17. R. Ryhage, S. Wikstrom and G.R. Waller, Anal. Chem. 37, 435 (1965).

18. J.T. Watson and K. Biemann, Anal. Chem. 36, 1135 (1964).

CHAPTER 3. ORGANIC GEOCHEMISTRY

Over the past hundred years organic geochemistry was concerned solely with the problem of petroleum formation. Even today petroleum geochemistry is centered around three aspects: first, the origin of petroleum hydrocarbons, second, the migration of these hydrocarbons and third, their final accumulation in the reservoir rock. In this chapter there are three sections. The first section deals with sediments and their relation to the origin of life as well as the origin of petroleum. The second and third sections deal with the chemical methods used to study the origin of life and the migration of petroleum, respectively.

With the aid of new analytical techniques organic geochemists are no longer limited to the study of petroleum. New areas have been opened in the study of chemical evolution, the origin of life and the possibility of extraterrestrial life. This last area will be discussed further in Chapter 9.

The association of organic matter with sediments has been studied for the past decade, particularly in ancient rocks. Some firm correlations between the morphological evidence and the organic matter present in the same rock permit a systematic search for chemical evidence of early life in the ancient sedments. Several classes of organic compunds have been reported in rocks, such as alkanes[1], fatty acids[2,3] and porphyrins[4]. These show promise as biological markers since they are evidently stable for long periods of time under geological conditions.

Among the hydrocarbons, the presence of a series of structures which consists of C_5H_8 isoprene units, has been cited as evidence for the biological origin of petroleum.

Isoprenoid compounds ranging from C_{14} (2,6,10-trimethylundecane) to C_{21} (2,6,10,14-tetramethylheptadecane) have been isolated from petroleum by Bendoraitis[5,6]. Other authors [7-11] have reported similar isoprenoid hydrocarbons from oil shales and ancient rocks.

In the present work the higher molecular weight isoprenoid hydrocarbons have been studied. Small scale adaptations of established separation and identification procedures have revealed the presence of C_{22} to C_{25} isoprenoid hydrocarbons.

1. Sediments

The existence and composition of organic compounds of possible biological origin in Precambrian rocks provide clues for the understanding of biological evolution and the origin of life. It is known that, given sufficient time, all organic material in sediments tends to be reduced either to aromatic condensates resembling graphite, or to aliphatic hydrocarbons. Elevated temperatures accelerate this process.

Most biological compounds have low thermal stability, so that they will not be preserved through geological time. However, some of the biogenic compounds, e.g., porphyrins, amino acids, fatty acids, ethers, ketones, are somewhat more stable so that diagenesis occurs slowly enough that some of the original material may be preserved. Porphyrins[12,13] and amino acids[14] have been investigated by others, and recently, fatty acids in ancient sediments have been reported by Van Hoeven _et al._[15]

A recent lake sediment, Florida Mud Lake (5000 years), the carbonaceous Soudan Shale from Minnesota (2.7×10^9 years), and the oldest known sediment on earth, the Onverwacht (3.4×10^9 years) are examined in this

section.

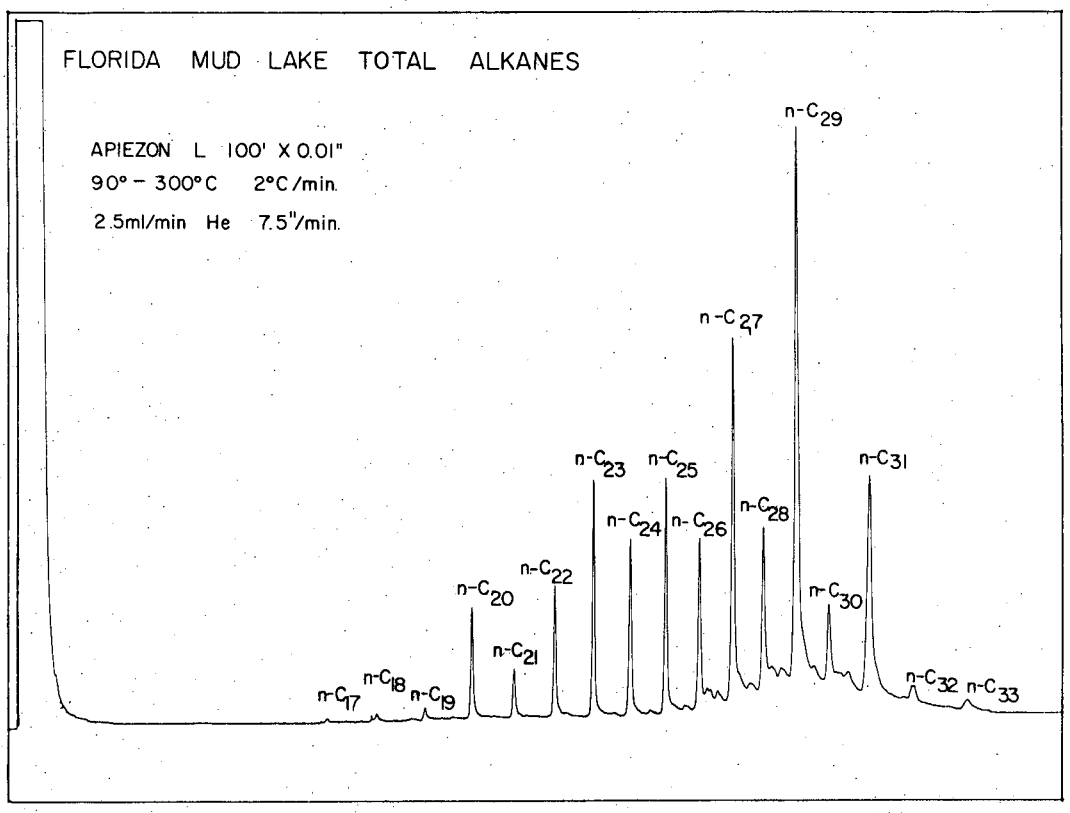
A. Mud Lake, Florida

The theory that algal oozes could give rise to oil shales is not a recent one.[9-11, 16-18] Evidence for this theory rests on the finding that algae have less cellulose and a correspondingly greater proportion of lipids than most plant material. In addition, the contemporary alga Botryococcus is present in microscopic remains in some organic oozes.[19] Since the algal ooze precursor theory rests primarily on geological and paleobotanical evidence, we have sought to complement this evidence by making a study of the constutuents of various genera of algae at the molecular level and comparing them with the organic constituents isolated and identified in tha algal ooze from a Florida Lake.

Bradley[20] has reported finding a lake in Florida that produces an organic ooze, predominantly algal in character, which he consider to represent the modern analogue of the precursors of rich oil shales, such as the Green River Shale. This organic ooze from Mud Lake, Florida, was analyzed for the aliphatic hydrocarbon content. A sample taken at a depth of 2 ft. below the mud-water interface showed a predominance of n-alkanes in the higher-molecular-weight region, n-C_{20} to n-C_{33}, particularly the n-C_{27}, n-C_{29} alkane, and n-C_{31} alkane, in contrast to our findings from the algae and the photosynthetic bacteria. A capillary gas chromatogram of the aliphatic hydrocarbons from the Mud Lake sample is shown in Fig. 1.

This laboratory[21] as well as other research groups[22-25] have found that the n-C_{17} hydrocarbon is a major hydrocarbon constituent of both the green algae and the blue-green algae. The normal hydrocarbons of

Figure 1: Gas chromatogram of total aliphatic hydrocarbons of Florida Mud Lake. A 100 ft. x 0.01 in. I.D. stainless steel column coated with Apiezon L was used. Helium flow rate 2.5 ml/min. Temperature was programmed at $2^{o}C$/min from $90^{o}C$ to $300^{o}C$ and was held isothermally at $300^{o}C$.


FLORIDA MUD LAKE TOTAL ALKANES
APIEZON L 100' X 0.01"
90° – 300°C 2°C/min.
2.5ml/min He 7.5"/min.
n-C17
n-C18
n-C19
n-C20
n-C21
n-C22
n-C23
n-C24
n-C25
n-C26
n-C27
n-C28
n-C29
n-C30
n-C31
n-C32
n-C33

XBL 670-6235

the rich oil shale from the Green River Formation exhibit a bimodal pattern with a maximum at n-C_{17} and at n-C_{29}, and are further characterized by a predominance of odd-numbered hydrocarbons over the even-numbered hydrocarbons. The occurrence of the n-C_{17} alkane in the Green River oil shale is consistent with the theory that algae, in part, give rise to the organic material of rich oil shale. Although the Florida Mud Lake ooze at the mud-water interface and below for about 6 inches consists wholly of minute fecal pellets that are made up exclusively of blue-green algae, there is no evidence for the presence of the n-C_{17} alkane as a major constituent of the Mud Lake sediment. The total heptane extract from this sediment exhibits a normal alkane distribution characteristic of a recent sediment.[26-28]. The occurrence of higher molecular weight hydrocarbons in the Mud Lake sediment might indicate a contribution from higher plant material. Pollen and spores are found in the Mud Lake sediments. A preliminary analysis in thes laboratory indicates the presence of n-C_{23} and n-C_{25} hydrocarbons as the major constituents of Ponderosa pine pollen. Nilsson et al.[29] have analyzed the constituents of pollen and have identified long-chain hydrocarbons, including the n-C_{25}, n-C_{27} and n-C_{29} alkanes. It is possible that wind-blown pollen contributed significantly to the hydrocarbons of higher molecular weight in these sediments.

B. Soudan Shale

The oldest carbonaceous Precambrian sediment known on the North American continent is the Soudan Shale of Northeastern Minnesota in the Lake Superior region. This shale has been studied extensively by Johns et al.[7] This study essentially follows their experimental method, so that the hydrocarbon distributions are similar to theirs. However, the

main purpose of this experiment was to look for evidence of early life, the very important biological markers 7- and 8-methylheptadecane. This study will be discussed further in Chapter 4.

Normal hydrocarbons (C_{13}-C_{21}) and isoprenoid hydrocarbons (C_{18}-C_{21}) were identified in the hydrocarbon fraction.

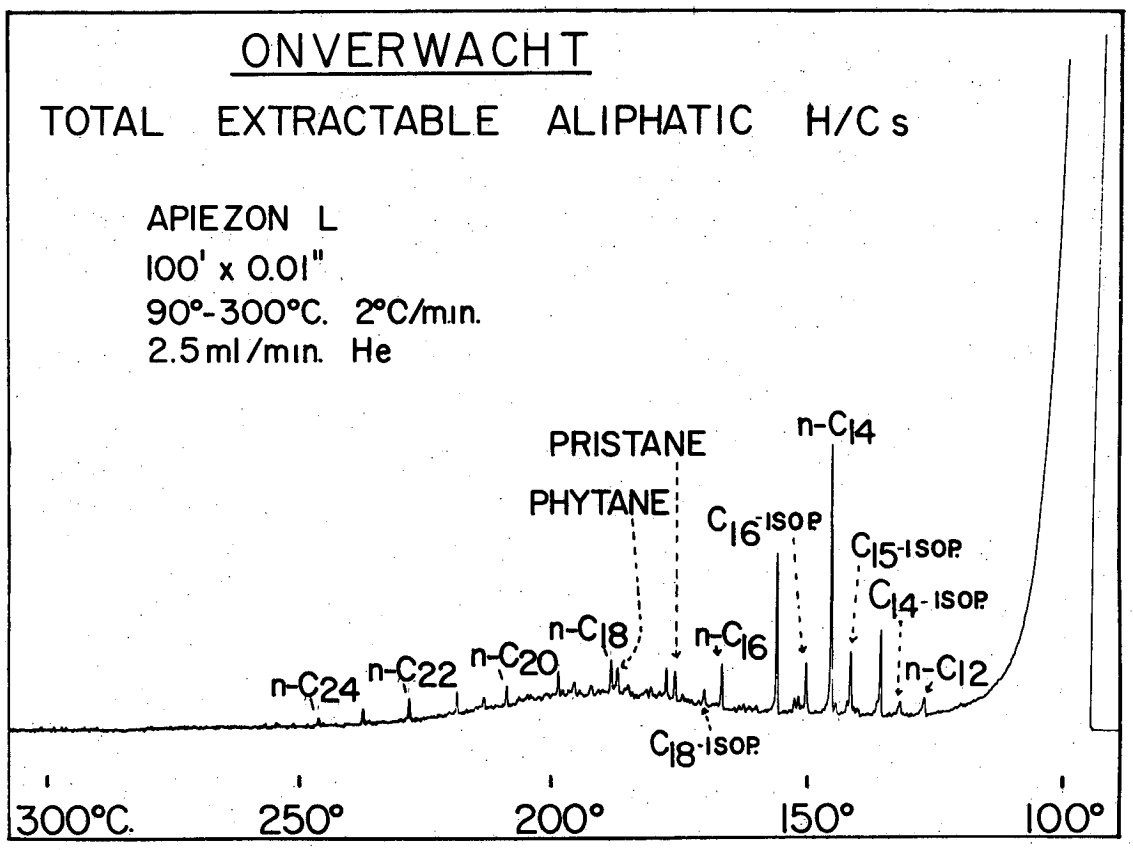
C. Onverwacht

A slightly metamorphosed sediment from the Onverwacht Series of the Swaziland System has been analyzed for aliphatic hydrocarbons and fatty acids. The Onverwacht is stratigraphically the lowest and therefore the oldest exposed strata in a series of sediments some 20,000 meters thick. Cloud[30] considers the Onverwacht to be 3.4 to 3.7 billion years old, and as such represents the oldest sedimentary rocks known on earth.

The preliminary chamical analysis showed 45.2% Si, 0.68% Al, 0.24% Fe, 3.8%C, and less than 0.01% for N, S, Cl and P.

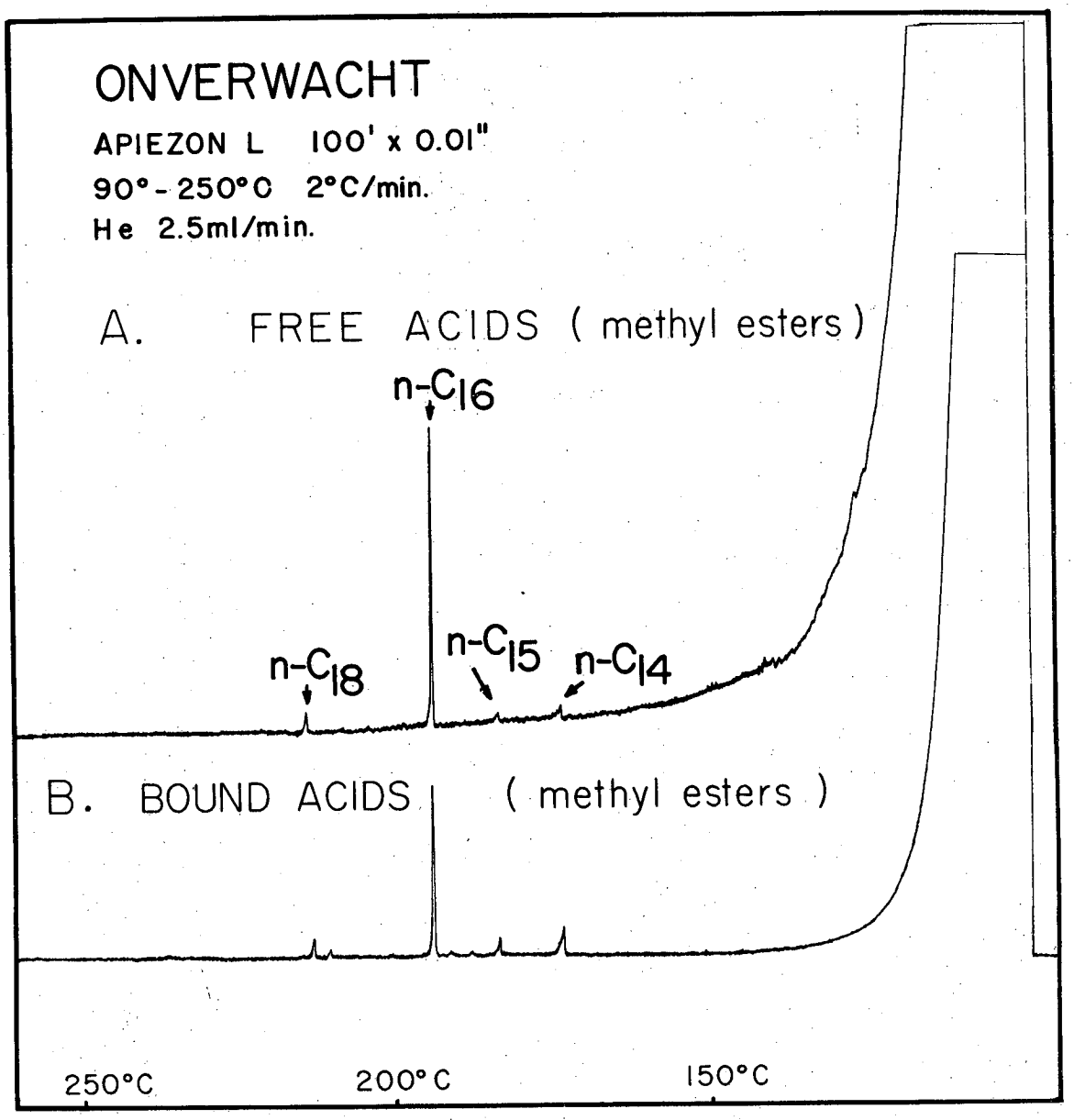
The outer surfaces of the sample were broken off and the remainder of the sample was thoroughly washed with solvent. The rock was pulverized to approximately 200 mesh with a disc mill. Following the procedure described in Chapter 2, four groups, free hydrocarbons, bound hydrocarbons, free fatty acids and bound fatty acids were isolated. All fractions were analyzed by combined capillary gas chromatography and mass spectrometry. Free aliphatic hydrocarbons (Fig. 2) represent 0.05 ppm wt./wt. of the rock, while there were no detectable bound hydrocarbons. In contrast, there were three times more fatty acids in the "bound" fraction as in the free fraction, representing 0.03 ppm and 0.01 ppm wt./wt. of the rock, respectively. The distribution of fatty acids with carbon number was identical in the bound and free fractions and is shown in Fig. 3.

Figure 2. Gas chromatogram of free hydrocarbons from Onverwacht. An Aerograph Model 204 gas chromatograph equipped with a flame ionization detector was used, with a 100 ft. x 0.01 in. I.D. stainless steel capillary column coated with Apiezon L. Helium pressure, 20 psi, no splitting, temperature programmed at $2^{o}C$/minute, from 90° to $250^{o}C$.


ONVERWACHT
TOTAL EXTRACTABLE ALIPHATIC H/C s
APIEZON L
100' x 0.01"
90°-300°C. 2°C/min.
2.5 ml/min. He
PRISTANE
PHYTANE
n-C14
C16-ISOP.
C15-ISOP.
C14-ISOP.
n-C18
n-C16
n-C12
n-C24
n-C22
n-C20
C18-ISOP.
300°C.
250°
200°
150°
100°

XBL692-4089

Figure 3. The top figure shows the gas chromatogram of the free fatty acids from Onverwacht. The bottom figure shows the gas chromatogram of bound fatty acids. All the conditions are identical to those in Fig. 1 except that the temperature was programmed from 90° to $250^{o}C$ and held isothermally.


ONVERWACHT
APIEZON L 100' x 0.01"
90°-250°C 2°C/min.
He 2.5ml/min.
A. FREE ACIDS (methyl esters)
n-C16
n-C18
n-C15
n-C14
B. BOUND ACIDS (methyl esters)
250°C
200°C
150°C

XBL 692-4109

Isoprenoid hydrocarbons represent a significant proportion of the alkanes isolated from the sediments which have been examined here, in fact, they tend to increase proportionaly with the age of the source material. The isoprenoid hydrocarbons have a wider range with increasing age. This could indicate that cracking and hydrogenation reactions were taking place at the time of geological origin and that isoprenoid hydrocarbons were generated. If isoprenoids are assumed to be indigenous to the sediments at the time of their deposition, that could strongly indicate that life had already started at that time. Contrary to the results of MacLeod[31], the Onverwacht does not have the odd-over-even predominance exhibited in the n-alkanes. It seems that only young sediments show a special odd-to-even preference. Other reports[2,32,33] also agree that no alternations were observed in Precambrian rocks.

The fact that fatty acids have been identified in the Onverwacht also favors a biological origin. The abundance of n-C_{14}, n-C_{16} and n-C_{18} is at present fully compatible with a biological origin since acids with these carbon numbers are the most abundant in living organisms and several young sediments.

2. Isoprenoid Hydrocarbons

This is the first isolation and identification of saturated hydrocarbons to C_{33} including isoprenoids to C_{25} in the Bell Creek Crude Oil, which is reservoired in Lower Cretaceous age sediments (135 x 10^6 years old), Powder River County, Montana.

A. Experimental

An alumina column (3 ft. x 0.5 in.) was prepared from 150 grams of activated neutral aluminum oxide and washed with 200 ml of n-heptane.

Two grams of crude oil were mixed with 100 grams aluminum oxide and transferred to the top of the column, after which it was eluted with 150 ml of n-heptane. The "total hydrocarbons" represented in this fraction show minimal ultraviolet absorption at 270 mμ. The alkanes were separated from alkenes by preparative thin layer chromatography on a 10% silver nitrate-silica gel plate using n-hexane as solvent. After the "total alkanes" were extracted from the silver nitrate-silica gel cut with dry isooctane, the normal alkanes were separated from the branched-cyclic alkanes with 5Å molecular sieve (O'Connor et al., 1962)[34]. A 50:1 weight ratio of sieve to sample was used. The sieve and sample were placed in a round-bottomed flask and refluxed in dry isooctane for two days. After centrifugation, the solution containing the branched-cyclic fraction was removed. The sieve was thoroughly extracted with hot isooctane for several hours and the washings were added to the branched-cyclic fraction. The isooctane-washed sieve, which contained the normal paraffins, was treated with a mixture of 20% HF and benzene for one hour. After separation of the layers, the benzene solution of n-alkanes was filtered, and the solvent was evaporated.

All fractions were analyzed on an Aerograph Model 204 gas chromatograph equipped with a capillary column which was coated in this laboratory. The gas chromatograms of "total aliphatic hydrocarbons", "normal alkanes", and "branched-cyclic alkanes" are shown in Figs. 4, 5 and 6 respectively. Three different columns, 100 ft. x 0.01 in. Apiezon L, 100 ft. x 0.01 in. Polysev, and 150 ft. x 0.01 in. SF-96 were used for this analysis. Coinjections of isoprenoid standards, C_{15}, C_{17}, C_{19}, C_{20} and C_{21} isoprenoid hydrocarbons, were made on all three columns. The locations of these standards coincide with the peaks labeled on Fig. 3.

The isoprenoids were isolated from the branched-cyclic fraction by preparative gas-liquid chromatography on an Aerograph Model A-90-P. The branched-cyclic fraction was chromatographed on a temperature-programmed 25 ft. x 1/4 in. 3% SE-30 column. Fractions were collected and rechromatographed isothermally on a 25 ft. x 1/8 in. HI-EFF-4B (butane-1,4-diol-succinate) column.

The final fractions collected from the polyester butandiol column were used for gas chromatograph-mass spectrometric analysis. A 100 ft. x 0.01 in. Apiezon L capillary column was used in the last step.

All mass spectra were taken using a combination of an Aerograph 204 gas chromatograph and an A.E.I. MS-12 mass spectrometer. The gas chromatographic oven temperature was programmed from 90° to 300°C at 2°C/min. with a helium flow rate of 2.5 ml/min. The effluent from the capillary column was split into two parts, 1.5 ml/min. going to the flame ionization detector and 1 ml/min. going through a 1 ft. x 0.002 in. heated stainless steel tube at 220°C directly into the ion source of the mass spectrometer. These spectra were determined at an ionizing voltage of 70 ev and an ionizing current of 50μA. The temperature of the ion source was 200°C. Each spectrum was scanned in three seconds. The spectra were recorded on an oscillograph recorder.

B. Results

The n-alkanes representing 2.75$\pm$ 0.2% of the Bell Creek Crude Oil range from C_{10} to C_{33} (Fig. 5). Identifications were made by mass spectral analyses and by coinjections with standard samples on three different gas chromatographic capillary columns; Apiezon L, Polysev, and SF-96.

Figure 4. Gas chromatogram of total aliphatic hydrocarbons of Bell Creek Crude Oil. A 100 ft. x 0.01 in I.D. stainless steel column coated with Apiezon L was used. Helium pressure; 20 psi; flow rate, 2.5 ml/min. An Aerograph 204 apparatus equipped with a flame ionization detector was used. Temperature was programmed at $2^{o}C$/min. from $90^{o}C$ to $300^{o}C$ and was held isothermally at $300^{o}C$.

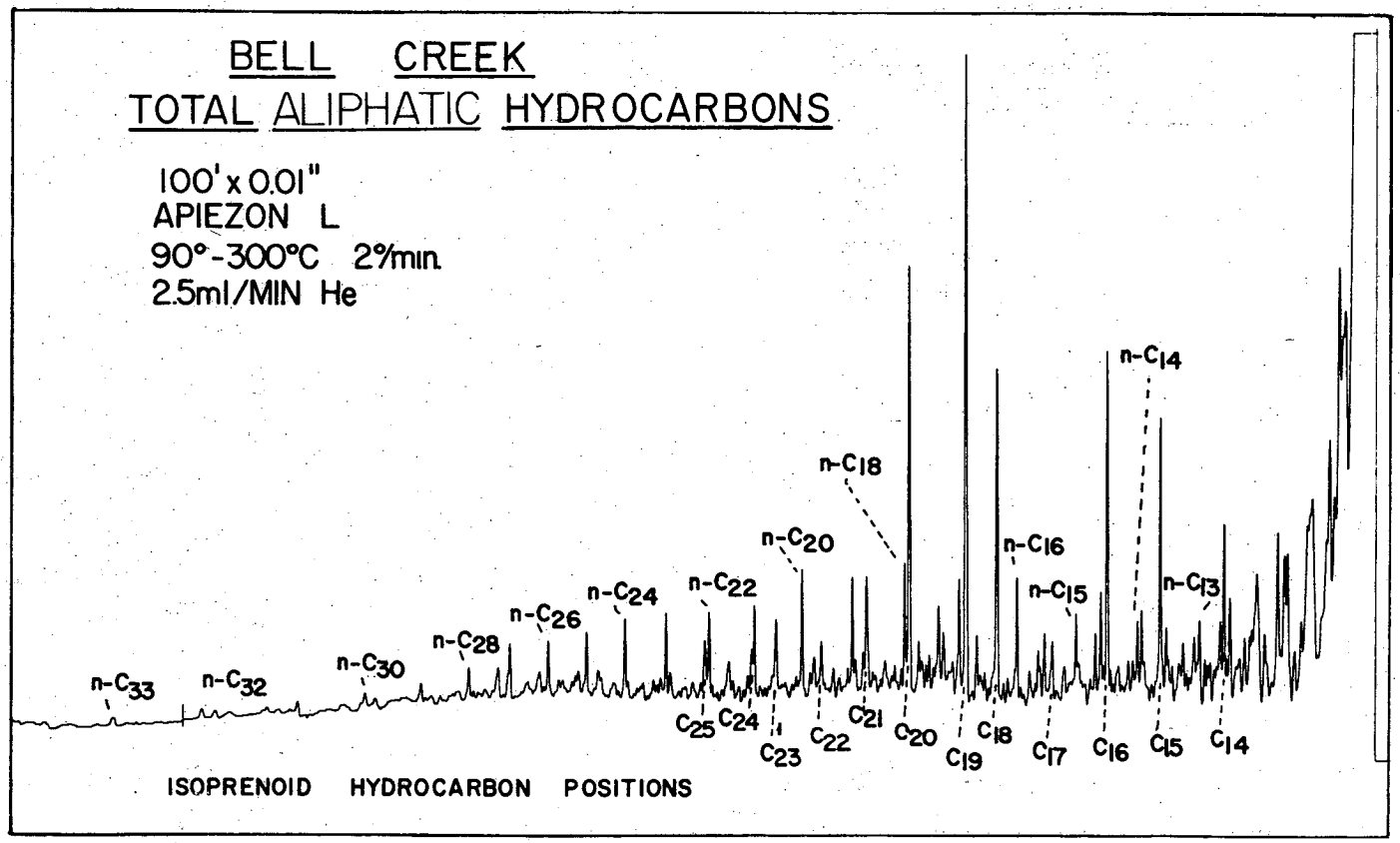


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Figure 5. Gas chromatogram of normal hydrocarbon fraction of Bell Creek Crude Oil. All conditions are the same as Fig. 1.

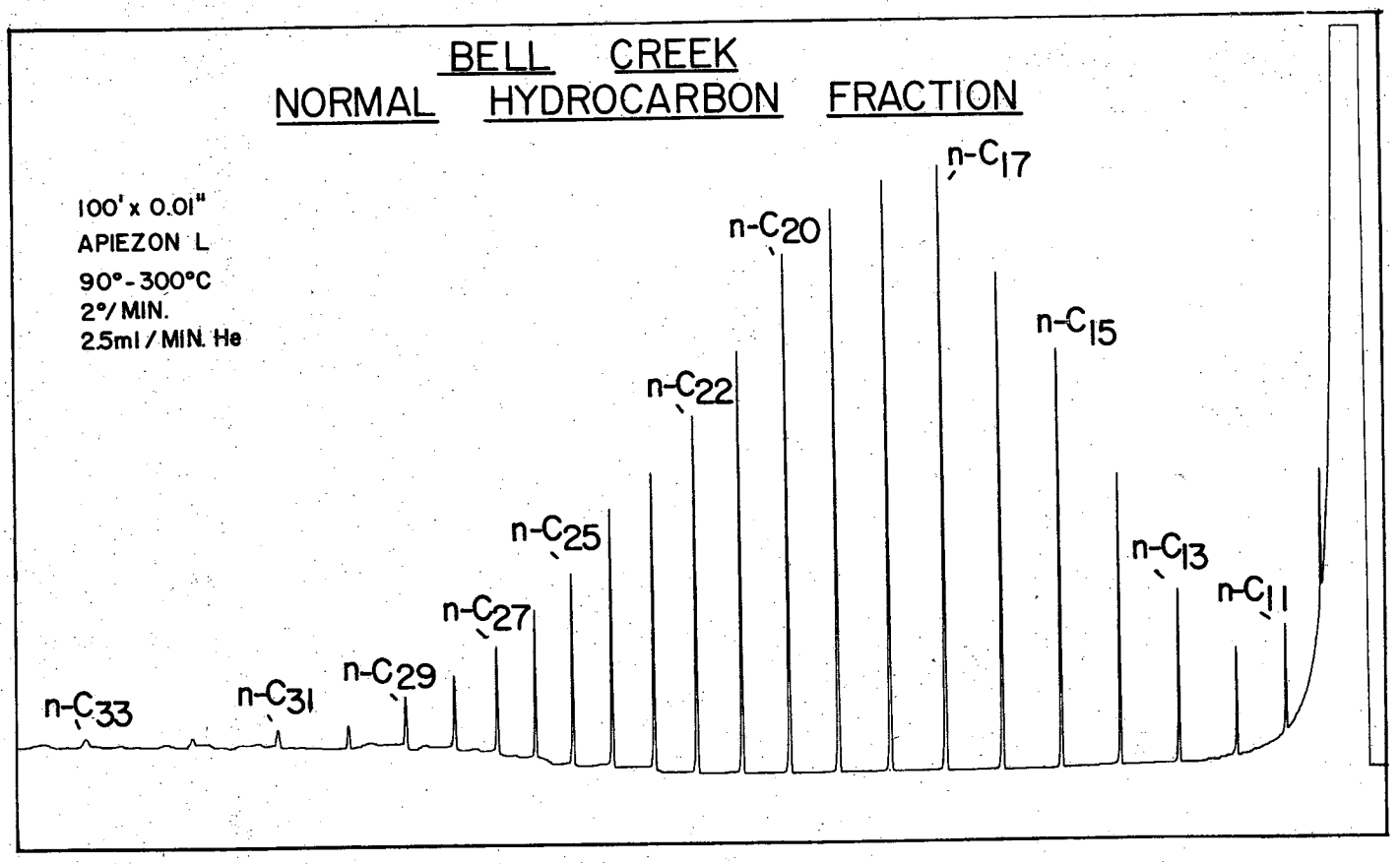

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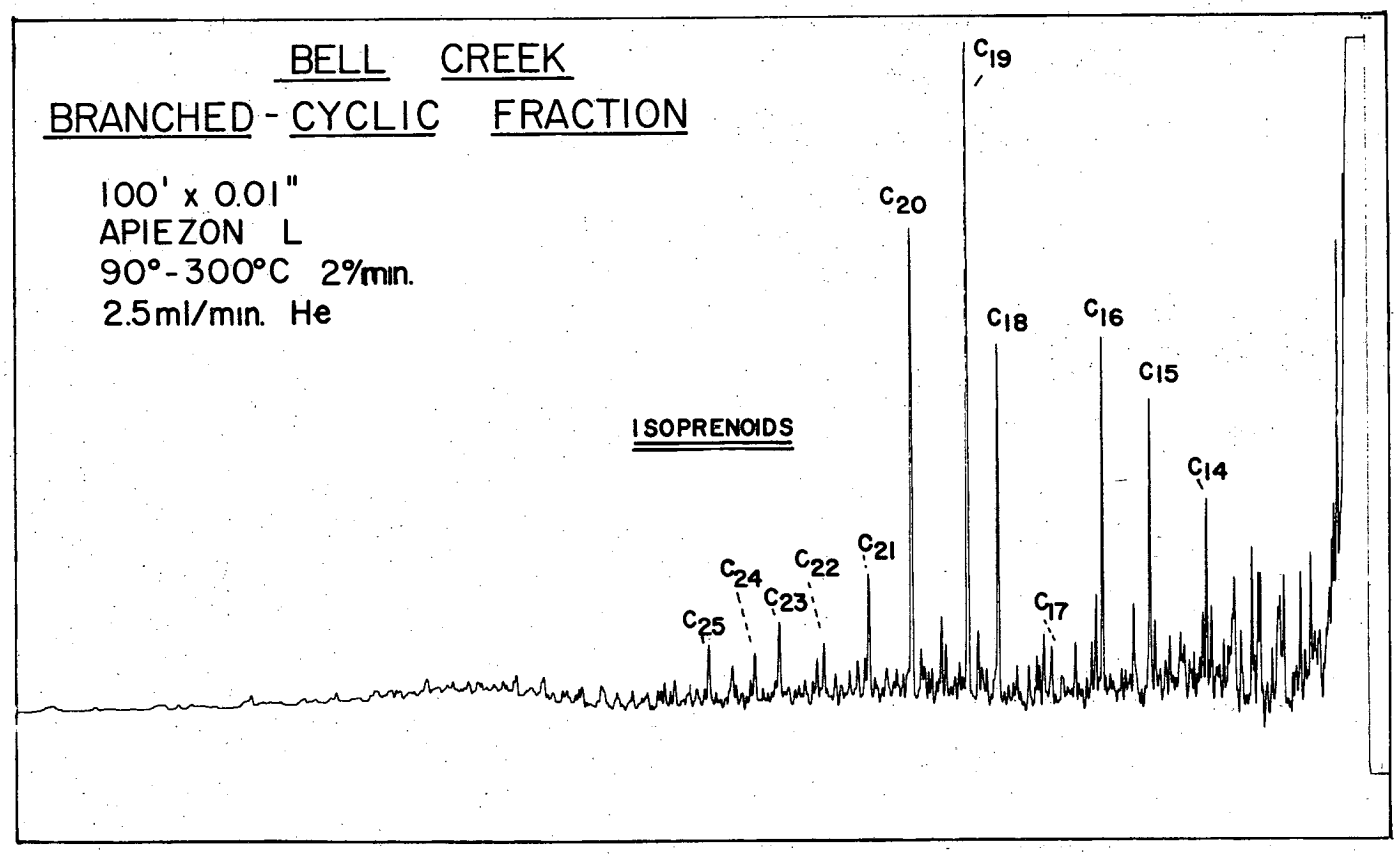
Figure 6. Gas chromatogram of the branched-cyclic alkane fraction of Bell Creek Crude Oil. All conditions are the same as Fig. 1.



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There was no significant predominance of odd over even carbon numbered n-paraffins. A similar observation was described by Bray (35). The most prevalent n-alkane was n-C_{17}. This result coincided with our earlier report (21, 36) (Han et al., 1968a, 1968b) that photosynthetic microorganisms, such as blue-green algae, green algae, and photosynthetic bacteria, contained more n-heptadecane than any other compound in the total hydrocarbon fraction.

Samples of C_{14} to C_{25} isoprenoid hydrocarbons were isolated from the branched-cyclic fraction (Fig. 6) and identified by their mass spectrometric fragmentation patterns. Figures 7-9 give the mass spectra of C_{14} to C_{25} isoprenoid alkanes isolated from this crude oil. For the C_{22}-C_{25} isoprenoids we suggest the structures I, 2,6,10,14-tetramethyloctadecane, II, 2,6,10,14-tetramethylnonadecane, III, 2,6,10,14,18-pentamethylnonadecane, and IV, 2,6,10,14,18-pentamethyleicosane respectively, based on their mass spectra. All of these compounds have regular head-to-tail condensations, having three methylene groups between two methyl branch sites.

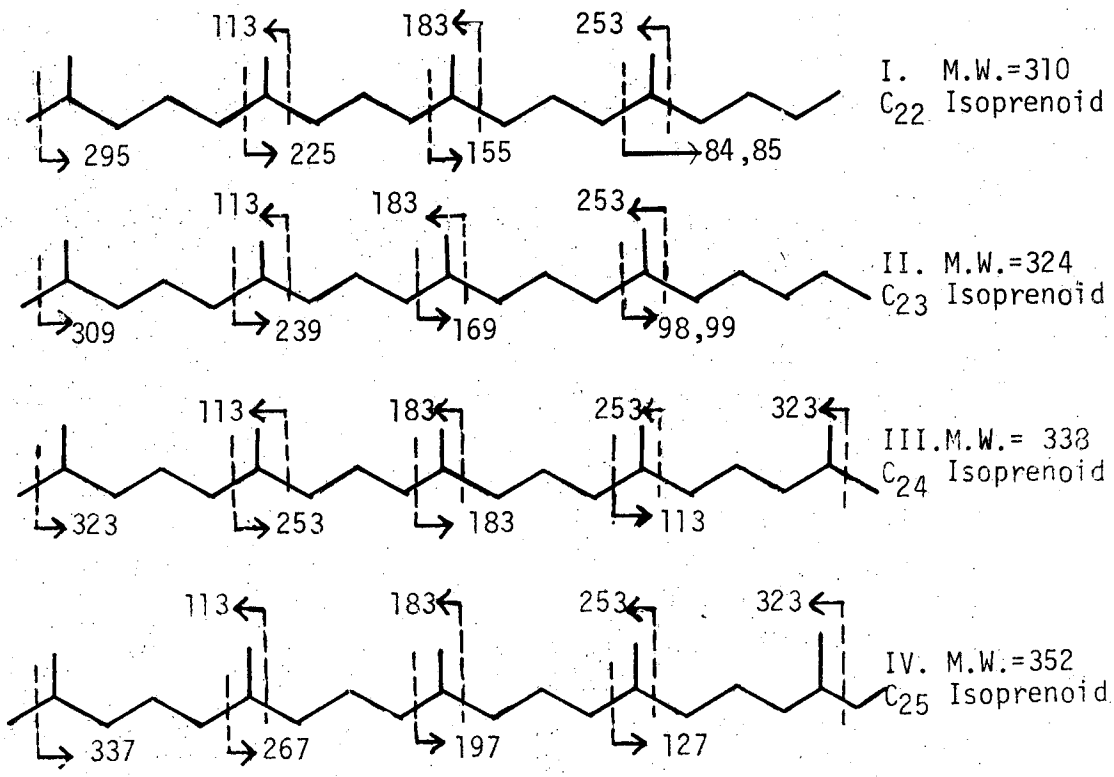

Figure 7. Mass spectra of C_{14} to C_{17} isoprenoid hydrocarbons. They were taken as the components were eluted from a 100 ft. x 0.01 in. I.D. Apiezon L capillary gas chromatographic column. The compounds were ionized by electron impact at 70 ev, the ion source temperature at 200°C, and an ionizing current of 50μA. Each peak was scanned within the range of 10-600 mass units in 3 sec. on an A.E.I. MS-12 mass spectrometer.

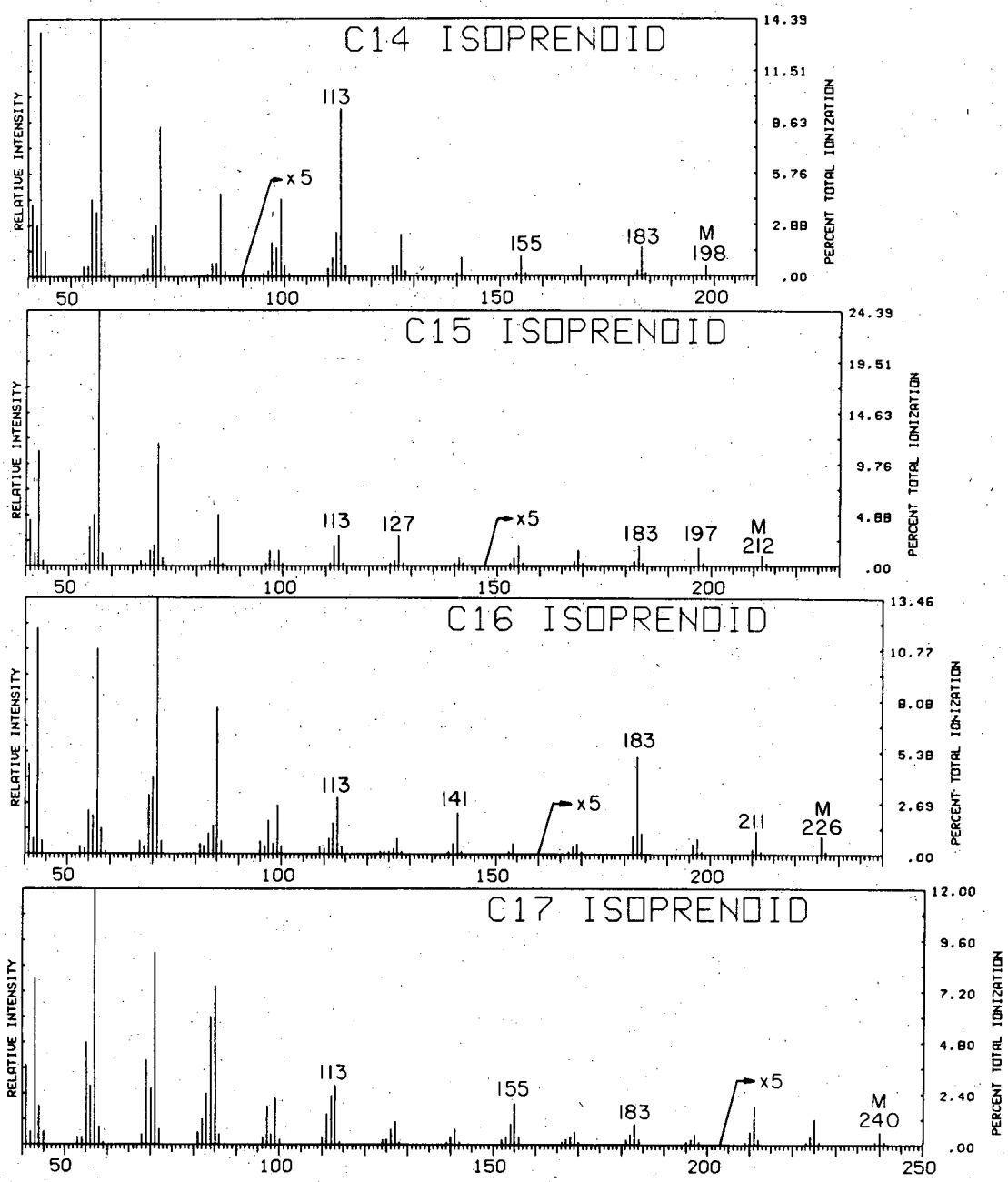

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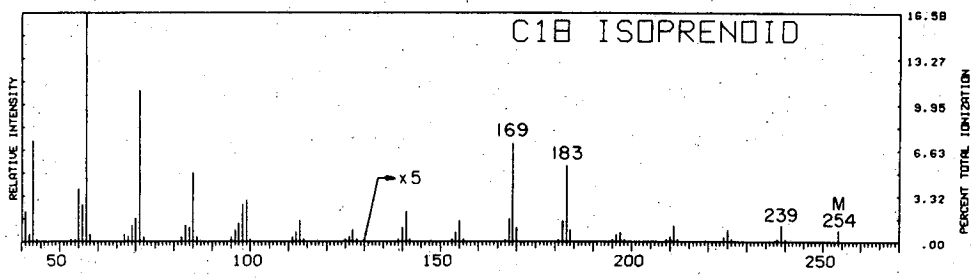
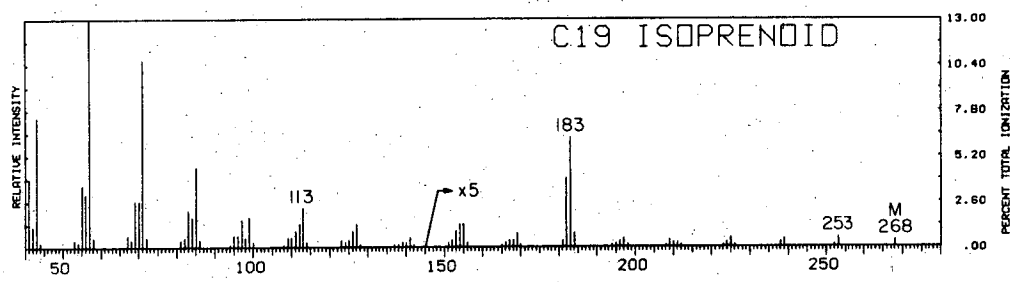
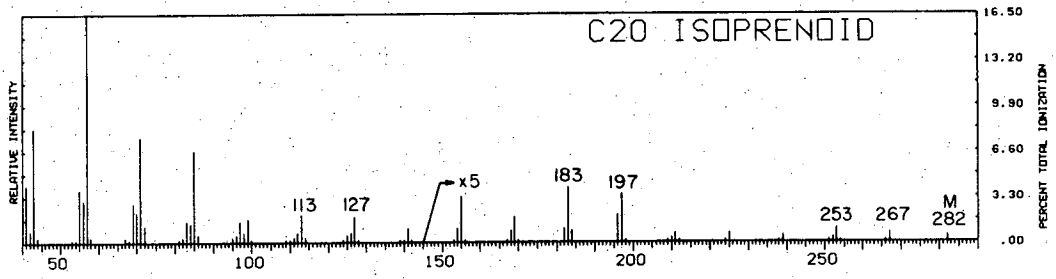
Figure 8. Mass spectra of C_{18} to C_{21} isoprenoid hydrocarbons. All conditions are the same as Fig. 4.







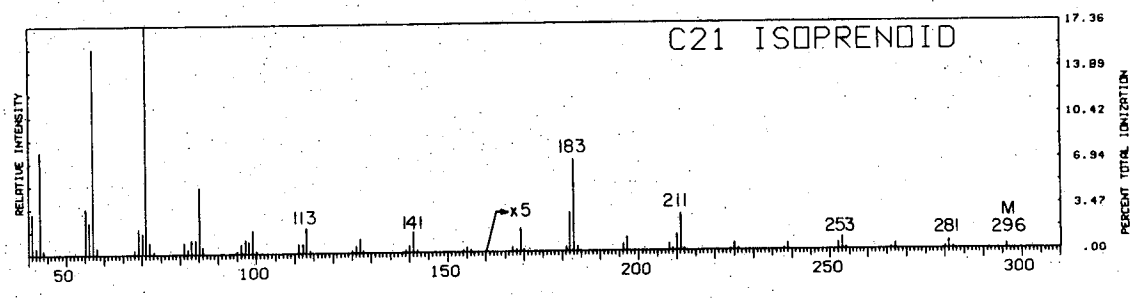


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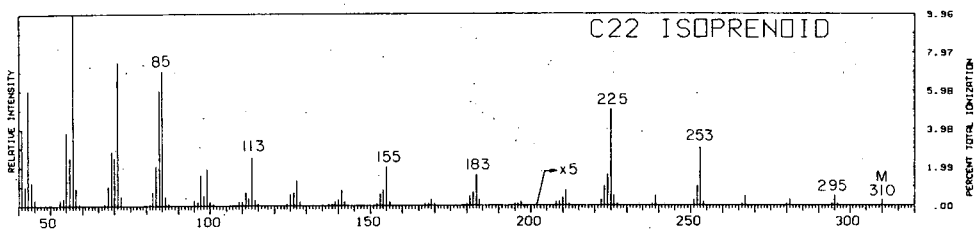
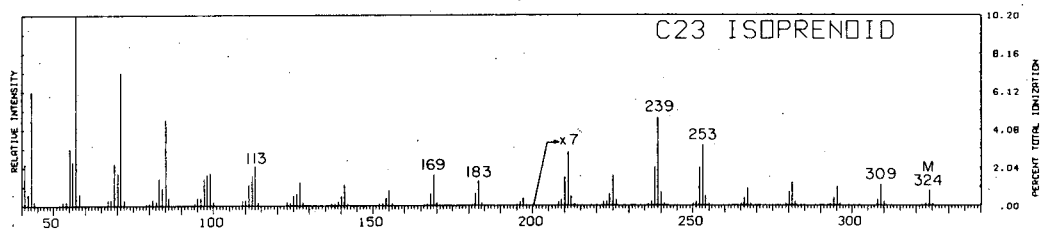
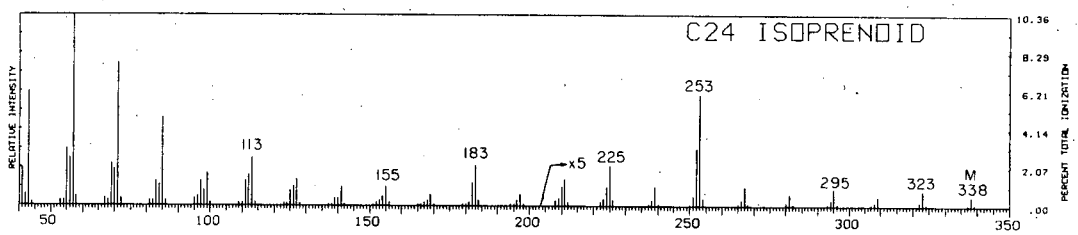
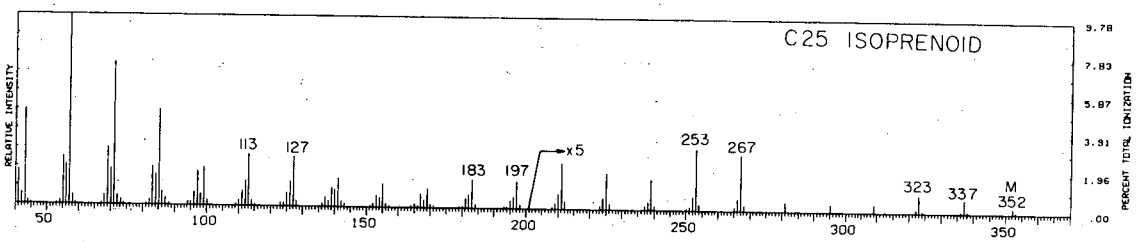
Figure 9. Mass spectra of C_{22} to C_{25} isoprenoid hydrocarbons. All conditions are the same as Figure 4.









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The C_{22} isoprenoid, I, exhibits six major fragments, C_8, C_{11}, C_{13}, C_{16}, C_{18} and C_{21} corresponding to fragment ions at m/e 113, 155, 183, 225, 253 and 295. Structures II, III and IV indicates the major fragment ions in Fig. 9. In Fig. 9, the spectrum for the C_{23} isoprenoid, a doublet at m/e 98 and 99 is observed; this phenomenon is described in our previous report (McCarthy et al., 1968) (37). When a fragment has a long straight chain the even peak C_nH_{2n} will be intensified. This phenomenon is also seen in Fig. 8, the fragmentation pattern of the C_{18} isoprenoid. The C_{23} isoprenoid (Fig. 9) does show the doublet at m/e 98 and 99, indicating the presence of a long straight chain. Thus, Fig. 9, the C_{23} isoprenoid, could not have the irregular structure, 2,6,10,14,17-pentamethyloctadecane, because it cannot produce a doublet at m/e 98 and 99. A double peak appears neither in the C_{24} nor C_{25} isoprenoid of Fig. 9, which eliminates the irregular structures; 2,6,10,14-tetramethyleicosane and 2,6,10,14-tetramethylheneicosane. The C_{19} and C_{20} isoprenoids, pristane and phytane, do not show doublets at m/e 112, 113 or m/e 126 and 127.

In Fig. 9, besides major fragments C_7, C_8, C_{12}, C_{13}, C_{17}, C_{18} and C_{22} for the C_{23} isoprenoid, significant fragments are also observed at C_{15} and C_{20}, which requires the charge to remain on the primary carbon atom. The same effect is seen in the C_{24} isoprenoids, which have major C_{16} and C_{21} fragments. This phenomenon is observed in high molecular weight hydrocarbons. After bond cleavage adjacent to a methyl branch the charge normally stays on the secondary carbon. If the primary fragment is sufficiently large, the charge can sometimes remain on the primary carbon fragment.

An estimate has been made of the abundance of isoprenoid hydrocarbons based on gas chromatographic data. This is shown in Table 1 by percentage weight of the Bell Creek Crude Oil:

Table 1. Isoprenoid content of the Bell Creek Crude Oil

	% (by wt.)		% (by wt.)
C_{14}	0.11 ± 0.02	C_{20}	0.46 ± 0.04
C_{15}	0.20 ± 0.02	C_{21}	0.09 ± 0.01
C_{16}	0.30 ± 0.03	C_{22}	0.04 ± 0.01
C_{17}	0.05 ± 0.02	C_{23}	0.06 ± 0.01
C_{18}	0.32 ± 0.03	C_{24}	0.03 ± 0.01
C_{19}	0.68 ± 0.05	C_{25}	0.05 ± 0.01

Identified isoprenoid alkanes constitute approximately 2.4% of the crude oil.

C. Discussion

In recent years many implications concerning the origin of petroleum were based on analytical data. The chemical evidence in favor of an organic origin of petroleum should not be regarded as a definite indication that all petroleum has originated biologically. Mueller[38] and Sylvester-Bradley[39] reported that hydrocarbons could be synthesized abiogenically both in the earth and in space. The analytical data on petroleum are summarized as follows:

1. Hulme[40] and Mair[41] have found that crude oils generally are optically active. This physical property is believed to be confined to biologically derived compounds. Thus the optical activity of petroleum has been taken as evidence of the biogenic origin of oil. The optical activity of petroleum is primarily

confined to the C_{14}-C_{18} and C_{27}-C_{30} carbon number ranges. This suggests that isoprenoids, sterols and triterpenoids could be the parent substances of such optically active compounds.

2. Silverman[42] studied the $^{13}C/^{12}C$ ratios in petroleum which are more similar to those of living organisms than to those of atmospheric CO_2 and carbonate rocks.
3. Some compounds isolated from petroleum have structural similarities to organic substances isolated from living organisms. Metal porphyrin complexes and some of the isoprenoid hydrocarbons are probably the diagenic products of chlorophyll.
4. The normal paraffinic hydrocarbons are the main constituents of petroleum.
5. Some petroleum exhibits a detectable predominance of n-alkanes with odd numbers of carbon atoms over those with an even number of carbon atoms. This is not only evidence for the biogenic origin of petroleum, but also suggests that the decarboxylation of fatty acids might be the mechanism by which the normal paraffins are generated.
6. The aromatic hydrocarbons, branched- and cyclo-alkanes are abundant in petroleum. Less branched structures are more common than highly branched ones. With increase in boiling range, the content of the cyclic and aromatic compounds increases. Most of the cyclo paraffins are related to cyclopentanes and cyclo-hexanes.
7. Porphyrin complexes and certain other compounds are unstable at temperatures higher than 250°C. This suggests the geochemical history of petroleum must have a relatively low temperature.

8. Olefins and other easily reduced compounds are found in very small amounts in petroleum. This could be interpretated as a formation of petroleum under a strongly reducing environment.
9. Whitehead and Breger[43] noted that, with the exclusion of prophyrins, no individual compound containing more than one hetero-atom has been isolated from petroleum to date.

The biological precursors of the C_{19} and C_{20} isoprenoids, pristane and phytane, have been the subject of much interest. These two compounds are considered to be derived from the phytyl side chain of the chlorophyll molecule, a precursor originally proposed by Bendoraitis et al. (5). In the Bell Creek Crude Oil, norpristane, pristane and phytane together account for over 60% of the total isoprenoids. Bendoraitis et al. (6) suggested that the sesquiterpenoid 2,6,10-trimethyldodecane (farnesane) and its degradation product 2,6,10-trimethylundecane also resulted from degradation of phytol in the source bed. However, another possibility should be considered. Since farnesol is the side chain of chlorophyll in some photosynthetic bacteria, for example Chlorobium (44) (Rapoport, 1961), it could be the precursor of farnesane. By a sequence of abiological diagenetic processes such as dehydration and reduction, it is possible to envisage the conversion of farnesol to farnesane. Again, oxidation, decarboxylation and reduction could lead to the C_{14} isoprenoid.

The hypothetical reduction and oxidative cleavage of polyenes, such as squalene and lycopene have been considered by McCarthy et al[45] and Bendoraitis et al.[6] Fig. 10 illustrates how C_{19} to C_{25} hydrocarbons might be derived from squalene by one bond cleavage. The isoprenoids found

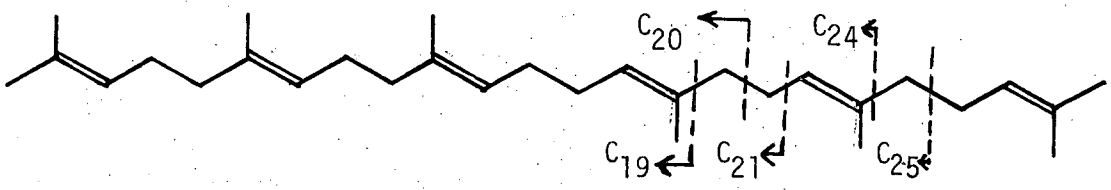
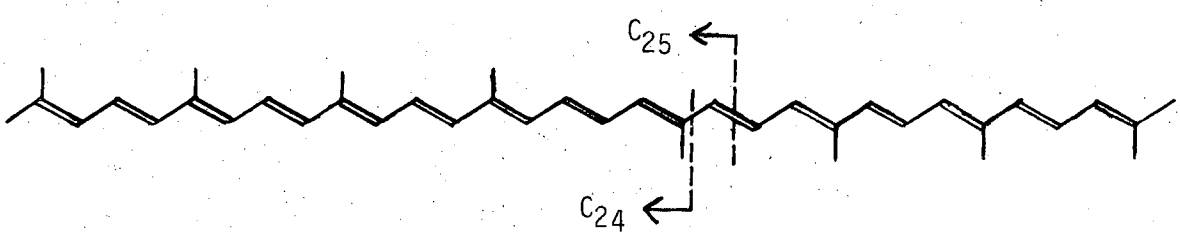


Fig. 10 Diagenetic pathway to "irregular" isoprenoid alkanes with squalene as precursor.

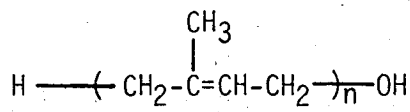
in crude oil and sediments are all "regular" isoprenoids containing only head-to-tail linkages. For example, only the regular C_{25} isoprenoid, 2,6,10,14,18-pentamethyleicosane has been found. The degradation products from squalene which contain four or five isoprenoids would be "irregular" isoprenoid alkanes. An "irregular isoprenoid" contains at least one head-to-tail or tail-to-tail linkage. In other words, a regular isoprenoid has three methylene groups between two methyl branch sites, but the irregular isoprenoid contains at least one unit which has either two methylene groups or four methylene groups between two methyl branch sites. The irregular C_{25} isoprenoid from squalene, 2,6,10,15,19-pentamethyleicosane is not present in any significant amount. Absence of the C_{19} isoprenoid, 2,6,10-trimethylhexadecane, the C_{20} isoprenoid, 2,6,10,15-tetramethylhexadecane, the C_{21} isoprenoid, 2,6,10,15-tetramethylheptadecane, the C_{23} isoprenoid, 2,6,10,15-tetramethylnonadecane, the C_{24} isoprenoid, 2,6,10,15-tetramethyleicosane, as well as the C_{25} isoprenoid, 2,6,10,15,19-pentamethyleicosane, indicates that squalene is not an important precursor of these isoprenoid hydrocarbons.

McCarthy et al.[45] suggested that lycopene (Fig. 11) could be the precursor of the C_{21} isoprenoid. If lycopene is an important precursor of lower molecular weight isoprenoid hydrocarbons, we would also expect it to be an



important precursor of the C_{24} and C_{25} isoprenoid alkanes. Fig. 11 shows how C_{24} and C_{25} isoprenoids can be derived from lycopene by one bond cleavage. Thus we would expect the irregular C_{24} isoprenoid, 2,6,10,14-tetramethyleicosane, and the irregular C_{25} isoprenoid, 2,6,10,14,19-pentamethyleicosane, if lycopene were the precursor. However, the C_{24} and C_{25} isoprenoids identified in the Bell Creek Crude Oil have the regular isoprenoid structures, i.e., 2,6,10,14,18-pentamethylnonadecane and 2,6,10,14,18-pentamethyleicosane. No significant amounts of irregular C_{24} and C_{25} isoprenoid hydrocarbons were observed. Thus, it appears that the common, naturally occurring isoprenoid hydrocarbons, squalene and lycopene, did not play an important role in the diagenetic pathway to isoprenoid alkanes isolated from this crude oil. Many other precursors (such as regular isoprenyl alcohols) are, however, available in living organisms. Regular head-tail-head-tail oligoterpenyl alcohols are rather common in nature.

Solanesol was isolated by Rowland *et al.* (46,47) from tobacco leaves where it was found partly as fatty acid esters. The structure of solanesol was confirmed by Fukawa et al. (48). Burgos and his co-workers (49) have



n=9 solanesol n=11 bactoprenol

n=6-9 betulaprenols n=20 dolichol

discovered dolichol in pig liver and Lindgren (50) has isolated a series of C_{30}-C_{45} terpenols, betulaprenols from birch wood. Thorne and his co-workers (51) gave the name bactoprenol to the most abundant lipid formed

by three species of Lactobacillus from mevalonic acid. The structure of this compound was confirmed as a C_{55} regular isoprenyl alcohol. A C_{110} isoprenoid substance has been identified as a metabolic product of Aspergillum fumigatus by Burgos et al. (52), while Hemming and his co-workers (53) detected a C_{50} isoprenoid alcohol in the spadix of Arum maculatum.

All isoprenoid alcohols have regular head-tail structures from end to end. These alcohols may be converted into high molecular weight isoprenoid alkanes by geochemical processes just as the low molecular weight compounds might be generated from a four-isoprenyl-unit alcohol, phytol. It is suggested that the initial degradation process has to take place by attack at the alcohol end of farnesol, phytol, betulaprenols, solanesol, etc. This could then be followed by reducing and thermal cracking mechanisms to give the C_{14} to C_{25} isoprenoid hydrocarbons found in the Bell Creek Crude Oil.

McCarthy (54,55) considered that the isoprenoid hydrocarbons might be formed from the polymerization of isoprene. The products could be expected to be present in hydrocarbon mixtures if they were derived by an abiogenic process which did not proceed stereospecifically.

Another abiogenic process could also be considered. Graphite could be reduced in geological time. In certain ways, to break its bonds the isoprenoid hydrocarbons would be expected. Fig. 12 shows that after the hydrogenation and cracking processes, pristane and phytane might be degradation products of a graphitic lattice. Assuming this reaction route is followed over geological time, isoprenoid hydrocarbons containing any number of carbon atoms could be produced. However, the question will immediately arise, why are the three and four unit isoprenoid hydrocarbons predominant in the product mixture? This leaves the whole mechanism very doubtful.

CHAPTER 3 REFERENCES

1. W.G. Meinschein, Space Science Rev. 2, 653 (1963).

2. P.H. Abelson and T.C. Hoering and P.L. Parker in "Advances in Organic Geochemistry, Colombo and Hobson (Eds.), Pergamon Press (1964) p. 164.

3. J.E. Cooper and E.S. Bray, Geochim. Cosmochim. Acta 27, 1113 (1963).

4. H.N. Dunning, in "Organic Geochemistry", I.A. Breger (ed) (1963) p. 367.

5. J.G. Bendoraitis, G.L. Brown and L.S. Hepner (1962) Isoprenoid hydrocarbons in petroleum. Anal. Chem. 34, 49-53.

6. J.G. Bendoraitis, B.L. Brown, and L.S. Hepner, Isolation and identification of isoprenoids in petroleum. World Petroleum Congress, Frankfort/Main, Germany, June 19-26. (1963).

7. R.B. Johns, T. Belsky, E.D. McCarthy, A.L. Burlingame, P. Haug, H.K. Schnoes, W. Richter and M. Calvin, The organic geochemistry of ancient sediments. Part II. Geochim. Cosmochim. Acta 30, 1191-1222. (1966).

8. E.D. McCarthy and M. Calvin, The isolation and identification of the C_{17} isoprenoid 2,6,10-trimethyltetradecane from a Devonian Shale: The role of squalene as a possible precursor. Tetrahedron 23, 2609-2619. (1967)

9. W.E. Robinson, J.J. Cummings, and G.U. Dineen, Changes in Green River oil-shale paraffins with depth. Geochim. Cosmochim. Acta 29, 249-258.(1965)

10. J.J. Cummings and W.E. Robinson, Normal and isoprenoid hydrocarbons isolated from oil-shale bitumen. J. Chem. Engng. Data 9, 304-307 (1964).

11. G. Eglinton and M. Calvin, Chemical fossils. Sci. Am. 216. 32-43. (1967).

12. W.G. Meinschein, E.S. Barghoorn and J.W. Schopf, Science 148, 461 (1965).

13. G.W. Hodgson, B. Hitchon, K. Taguchi, B.L. Baker, and E. Peake, Geochim. Cosmochim. Acta 32, 737 (1968).

14. J.W. Schopf, K.A. Kvenvolden and E.S. Barghoorn, Proc. Nat. Acad. Sci. 59, 639 (1968).

15. W. Van Hoeven, J.R. Maxwell and M. Calvin, Geochim. Cosmochim. Acta, 33, 877 (1969).

16. M.D. Zalesskey, Com. Geol. Bull., 33, 495(1914); M.D. Zalesskey, Soc. Paleont. Russie Annuaire, 1, 25 (1916).

17. R. Thiessen, U.S.G.S. Prof. Paper, 132, 126 (1925).

18. D. White, in Treatise on Sedimentation, ed. W.H. Twenhofel (Baltimore: Williams & Wilkins, 1926), pp. 289-304.

19. K.B. Blackburn, and B.N. Temperley, Trans. Roy. Soc. Edin., 58, 841 (1936).

20. W.H. Bradley, Geol. Soc. Am. Bull., 77, 1333 (1966).
M.T.J. Murray, A. McCormick, and G. Eglinton, Science, 157, 1042 (1967).

21. J. Han, E.D. McCarthy, M. Calvin, and M.H. Benn, J. Chem. Soc. (c) 2785 (1968).

22. R.C. Clark, Jr. and M. Blumer, Limnol. Oceanog., 12, 79 (1967).

23. R.C. Clark, Jr., M.S. thesis, Massachusetts Institute of Technology (1966).

24. I.M. Heibron, E.G. Parry, and R.F. Peters, Biochem. J., 29, 1376 (1935).

25. J. Oró, T.G. Tornabene, D.W. Nooner, and E. Gelpi, J. Bacteriol., 93, 1811 (1967).

26. E.D. Evans, G.S. Kenny, W.G. Meinschein, and E.E.Bray, Analy. Chem., 29, 1858 (1957).

27. K.A. Kvennolden, Bull. Am. Assoc. Petrol. Geol., 46, 1643 (1962).

28. N.P. Stevens, E.E. Bray, and E.D. Evans, Bull. Am. Assoc. Petrol. Geol., 40, 975 (1956).

29. M. Nilsson, R. Ryhage, and E. Von Sydow, Acta Chem. Scan., 11, 634 (1957).

30. P.E. Cloud, Personal Communication, Dept. of Geology, University of Calif. Santa Barbara, Calif.

31. W.D. MacLeod, J. Gas Chromatog. 6, 591 (1968).

32. J. Oró, D.W. Nooner, A. Zlatkis, S.A. Wikstrom, and E.S. Barghoorn, Science 148, 77 (1965).

33. E.E. Bray and E.D. Evans, Bull. Am. Assn. Petrol. Geol. 49, 248 (1965).

34. J.G. O'Connor, F.H. Burrow, and M.S. Norris, Anal. Chem. 34, 82(1962).

35. E.E. Bray and E.D. Evans, Distribution of n-paraffins as a clue to recognition of source beds. Geochim. et Cosmochim. Acta 22, 2-15 (1961).

36. J. Han, E.D. McCarthy, W. Van Hoeven, M. Calvin and W.H. Bradley. Organic geochemical studies II: A preliminary report on the distribution of aliphatic hydrocarbons in algae, in bacteria, and in a Recent lake sediment. Proc. Natl. Acad. Sci. 59, 29-33. (1968a).

37. E.D. McCarthy, J.Han, and M. Calvin, Hydrogen atom transfer in mass spectrometric fragmentation patterns of saturated aliphatic hydrocarbons. Analyt. Chem. 40, 1475-1480. (1968)

38. G. Mueller, World Petrol. Cong., Proc., 6th Frankfurt 1, 383 (1963).

39. P.C. Sylvester-Bradley, and R.I. King, Nature, 198, 728 (1963).

40. R.E. Hulme, St. Bonaventure Sci. Studies, 6, 125 (1938).

41. B.J. Mair, C.B. Willingham, and A.J. Streiff, J. Res. Natl. Byr. Std. 21, 581 (1938).

42. S.R. Silverman, Isotopic and Cosmic Chemistry, Eds Craig, Miller, Wasserburg, North-Holland, Amsterdam, p. 92 (1964).

43. W.L. Whitehead and I.A. Breger, Organic Geochemistry, Ed. Breger, I.A., Pergamen, London, P. 248 (1963).

44. H. Rapoport and H.P. Hamlow. Chlorobium chlorophyll-660. The esterifying alcohol. Biochem. and Biophys. Res. Comm. 6, 134-137. (1961).

45. E.D. McCarthy, W. Van Hoeven and M. Calvin. The synthesis of standards in the characterization of a C_{21} isoprenoid alkane isolated from Precambrian sediments. Tetrahedron Letters, 4437-4442. (1967b).

46. R.L. Rowland, P.H. Latimer and J.A. Giles. Flue-cured tobacco I. Isolation of solanesol, an unsaturated alcohol. J. Amer. Chem. Soc. 78, 4680-4683.

47. R.L. Rowland and J.A. Giles. Flue-cured tobacco V. Polyisoprenoid compounds. Tobacco Science 4, 29-32. (1960).

48. H. Fukawa, M. Toyodo, T. Shimizu and M. Murohashi. Isolation of new isoprenyl alcohols from silkworm feces. Tetrahedron Letters 49, 6209-6213. (1966).

49. J. Burgos, F.W. Hemming, J.F. Pennock and R.A. Morton. Dolichol: A naturally-occurring C_{100} isoprenoid alcohol. Biochem. J. 88, 470-482. (1963).

50. B.O. Lindgren. Homologous aliphatic C_{30}-C_{45} terpenols in birch wood. Acta Chem. Scand. 19, 1317-1326. (1965).

51. K. Thorne and E. Kodicek. The structure of Bactoprenol, a lipid formed by Lactobacilli from mevalonic acid. Biochem. J. 99, 123-127. (1966).

52. J. Burgos, P.H.W. Butterworth, F.W. Hemming and R.A. Morton. The biosynthesis of long-chain polyisoprenoid alcohol by Aspergillus fumigatus, Fresenius. Biochem. J. 91, p. 22.

53. F.W. Hemming, R.A. Morton and J.F. Pennock. Constituents of the unsaponifiable lipid fraction from the spadix of Arum maculatum. Proc. Roy Soc. B158, 219-310. (1963).

54. E.D. McCarthy, A Treatise in Organic Geochemistry, Ph. D. Thesis, Univ. of California, Berkeley, 1967.

55. E.D. McCarthy and M. Calvin, Nature, 216, 642 (1967).

CHAPTER 4. A NEW BIOLOGICAL MARKER, 7-AND 8-METHYLHEPTADECANE

1. Introduction

The lipids have probably received the most attention of all the biological markers, because they are more stable than any other organic compound. Among the lipids, the presence of isoprenoid hydrocarbons has been found and studied extensively during the past ten years. However, it was pointed out by McCarthy et al.[1] that the isoprenoid molecules could also possibly be polymerized from small isoprene units by an abiogenic process. At this stage the search for the new biological markers could give additional information about biological and abiological origin.

The analysis of the heptane eluate, primarily by combined capillary gas chromatography and mass spectrometry, revealed the presence of a series of normal hydrocarbons, ranging in carbon number from n-C_{15} to n-C_{20}, with the n-C_{17} hydrocarbon being the predominant member for each species. In addition, C_{18} branched alkanes occurred in all the blue-green algae, which have heretofore not been found elsewhere.

The structural identity of the C_{18} branched alkanes seemed to us of particular significance because they appeared to occur uniquely in blue-green algae and were absent from certain photosynthetic and non-photosynthetic bacteria. These hydrocarbons also were not found in the green algae, more advanced than the blue-green algae but still relatively primitive.

2. Physical Measurements

In an early report[2] a tentative *Nostoc* branched C_{18} alkane structure, 7,9-dimethylhexadecane was proposed based purely on mass spectral evidence.

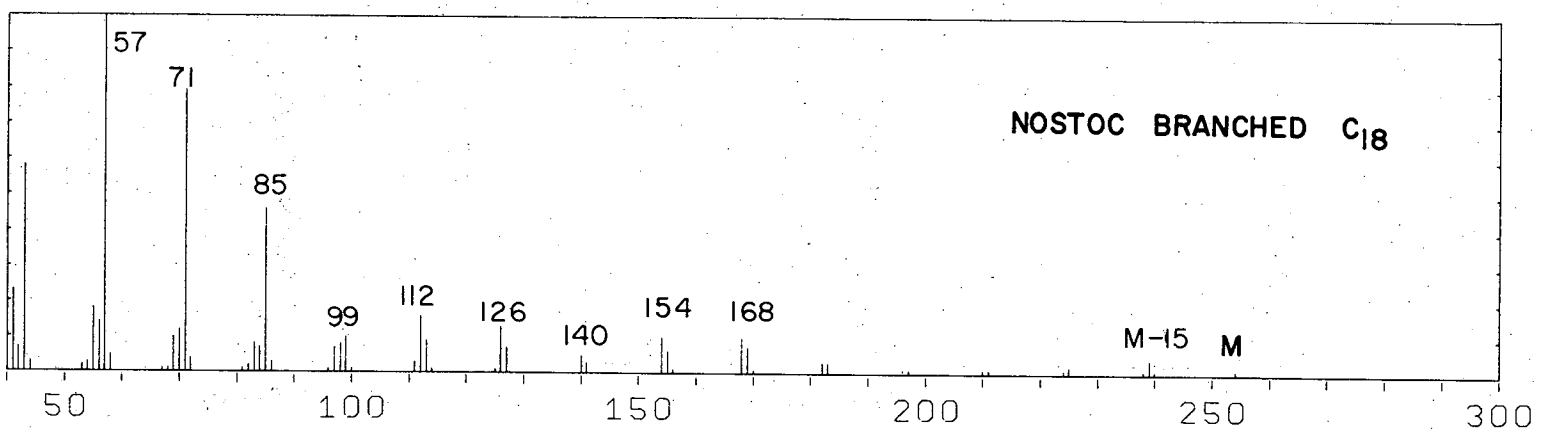
This chapter will conclusively identify the structure of the C_{18} hydrocarbon and show that our original proposal of a dimethyl hydrocarbon is in fact incorrect.

The mass spectrum of the C_{18} branched alkane isolated from Nostoc is shown in Figure 1. The unique feature of this spectrum is the occurrence of major "doublets" of peaks at C_8, C_9, C_{11} and C_{12} fragment ions, corresponding to m/e 112, 113, m/e 126, 127, m/e 154, 155, and m/e 168, 169; in each fragment the even peak is more intense than the odd peak. The feature in the mass spectral fragmentation patterns of other branched hydrocarbons will be discussed in more detail in Chapter 8. If 7,9-dimethylhexadecane was the structure of the Nostoc branched-C_{18} hydrocarbon, then the only anomalous feature of the mass spectra which might be inconsistent with that structure was the relative intensity of the 154 and 168 peaks relative to 155 and 169. In keeping with this designation, the infrared spectrum of the C_{18} branched alkane from Nostoc showed no absorption corresponding to the presence of gem-dimethyl groups in the molecule (Fig. 2)[3]. This spectrum was obtained using a micro-beam condenser and a 50 μg sample of hydrocarbon. The nuclear magnetic resonance spectrum of the hydrocarbon was obtained with some difficulty on a 500 μg sample. The integrated spectrum indicated a methyl to methylene proton ratio of approximately 1:2.5. Finally, the capillary GLC retention time (Fig. 3) indicated that the structure probably contained no more than one or two methyl groups.

3. Synthesis Procedure

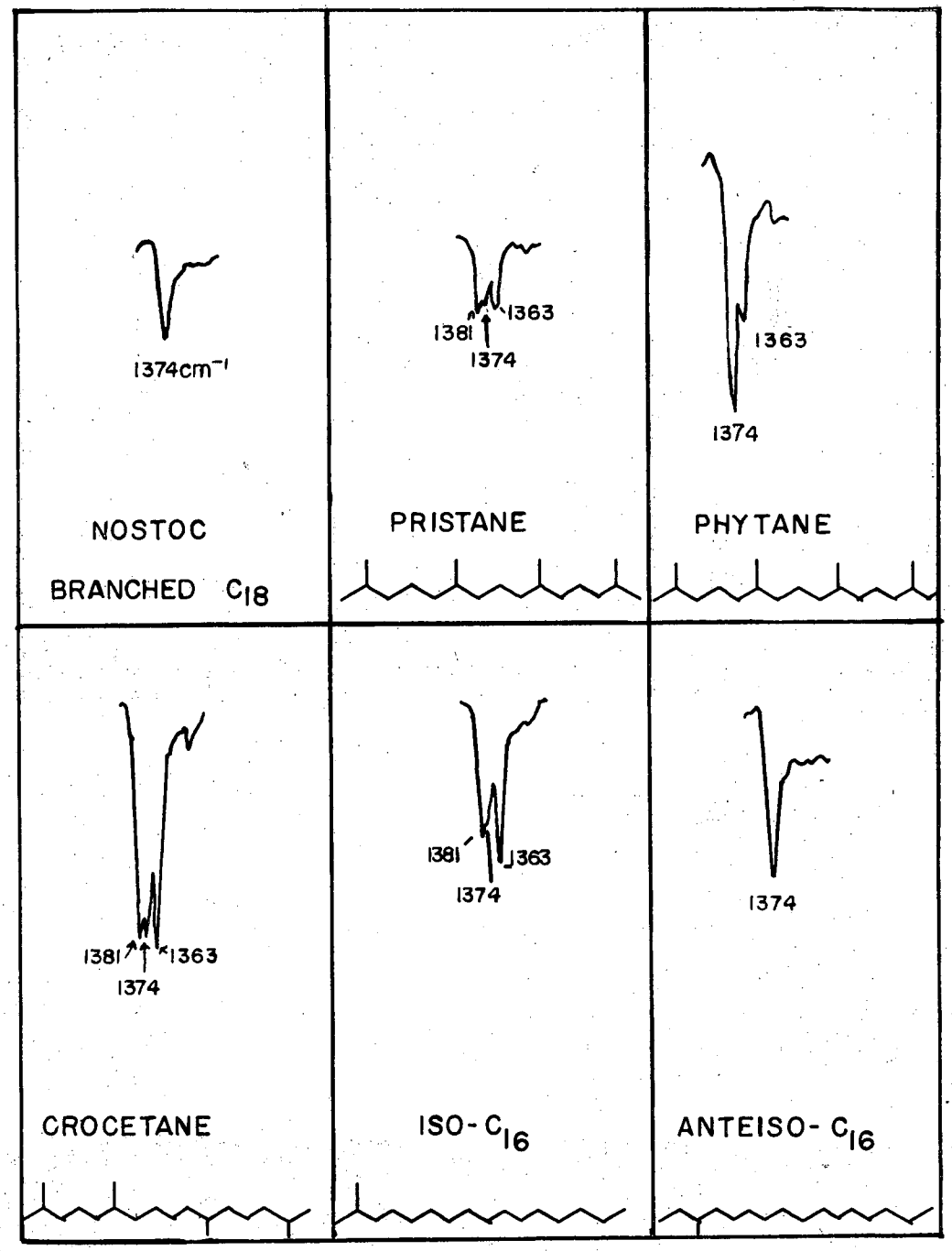
With this spectral evidence we set about to confirm the structure of the hydrocarbon by synthesis. Our first synthesis was non-stereospecific

Figure 1: Mass spectrum of the Nostoc branched-C_{18} alkane obtained using the MS-12. The compound is ionized by electron impact at 70ev.



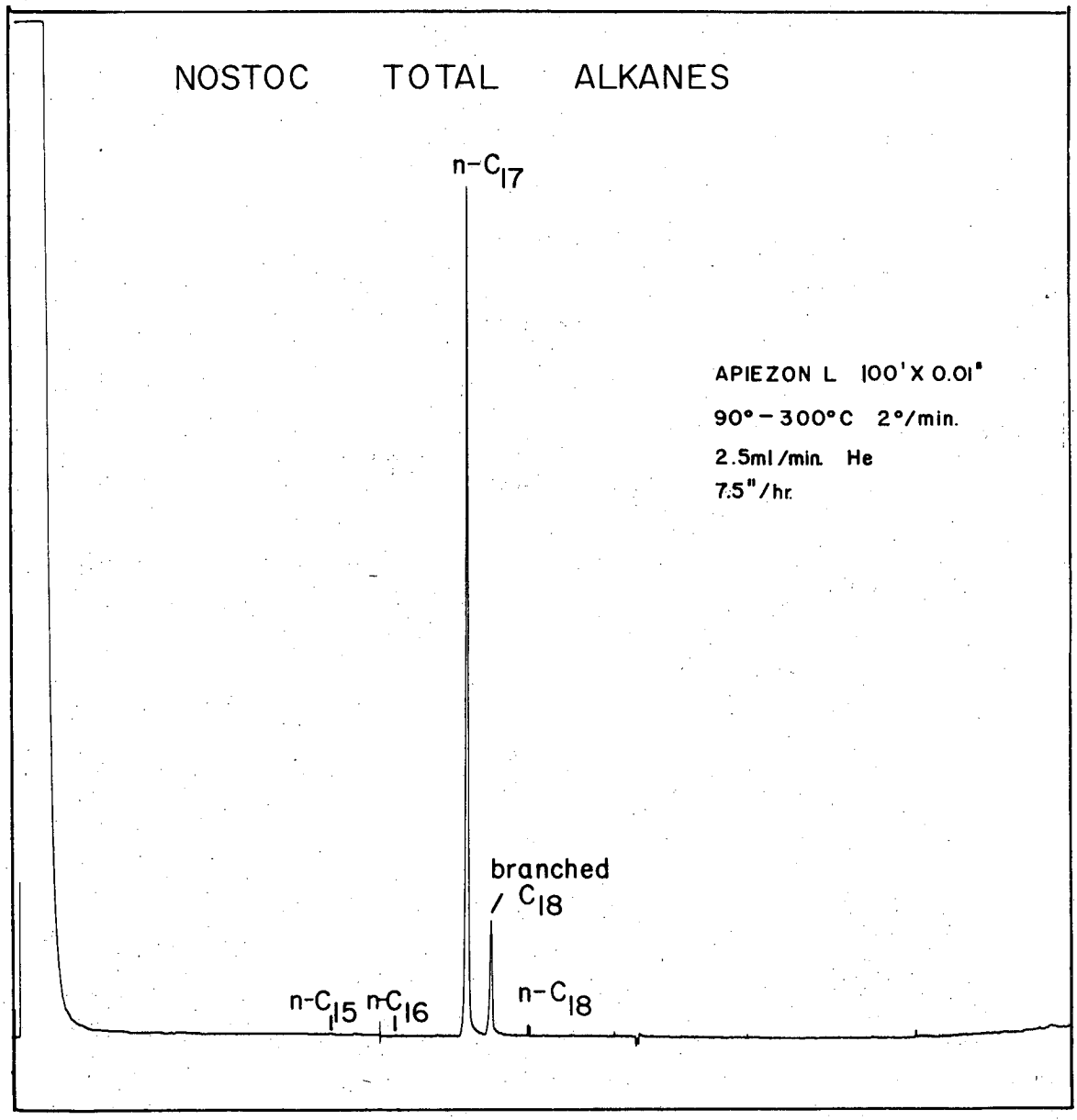
XBL 6710-6232

Figure 2: IR absorption bands are obtained with a Perkin-Elmer 257 Spectrophotometer equipped with a micro cell and beam condensor. The compounds with or without gem-dimethyl groups showed different absorption bands.


1374cm⁻¹
NOSTOC
BRANCHED C18
1381
1363
1374
PRISTANE
1363
1374
PHYTANE
1381
1363
1374
CROCETANE
1381
1363
1374
ISO-C16
1374
ANTEISO-C16

XBL 682-4037

Figure 3: Gas chromatographic separation of Nostoc hydrocarbon mixture using an Aerograph-204 instrument. A stainless steel tubing 100 ft. x 0.01 in., coated with Apiezon L. Helium pressure 20 psi; no split. Temperature was programmed at $2^{o}C$/min. from $90^{o}C$ to $300^{o}C$.


NOSTOC TOTAL ALKANES
n-C17
APIEZON L 100' X 0.01"
90° – 300°C 2°/min.
2.5ml/min. He
7.5"/hr.
branched
/ C18
n-C15
n-C16
n-C18

XBL 670-6225

and produced both diastereoisomers in equimolar amounts. The synthetic outline is shown in Figure 4. Two aspects of this synthesis deserve some consideration. In the first place the conversion of 7,9-dimethyl-8-hexadecanol into the corresponding ketone produces two compounds, which have identical mass spectra (Fig. 5) and infrared spectra, and which can be separated by preparative gas chromatography. The confirmation of the fact that these two components were the diastereoisomers of the C_{18} ketone was obtained by first isolating the individual components in turn and then converting each of them into an equimolar diastereoisomeric mixture again, by refluxing with 10% KOH in ethanol for two hours. The purity of one ketone is shown in Fig. 6, checked by gas chromatography. The gas chromatogram of epimerization products is shown in Fig. 7. Secondly, the final step of the synthesis, a Wolff-Kishner reduction, was carried out with some difficulty and gave a poor yield. However, there were two major C_{18} hydrocarbon components in the crude product which could be separated by capillary gas chromatography and which were identified as the two diastereoisomers of 7,9-dimethylhexadecane and two mass spectra are shown in Fig. 8. To my knowledge this represents the first separation of the long chain diastereoisomers of an acyclic hydrocarbon by capillary gas-liquid chromatography (Fig. 9). To determine which component corresponded to the erythro diastereoisomer (meso-like) and which to the threo diastereoisomer (dl-like) we synthesized the pure erythro diastereoisomer starting from cis-dimethylcyclohexanone. The synthetic outline is shown in Figure 10. Coinjection of this diastereoisomer with the mixture of diastereoisomers showed that the erythro isomer had the shorter retention time.

Figure 4. Non-stereospecific synthesis of 7,9-dimethylhexadecane.

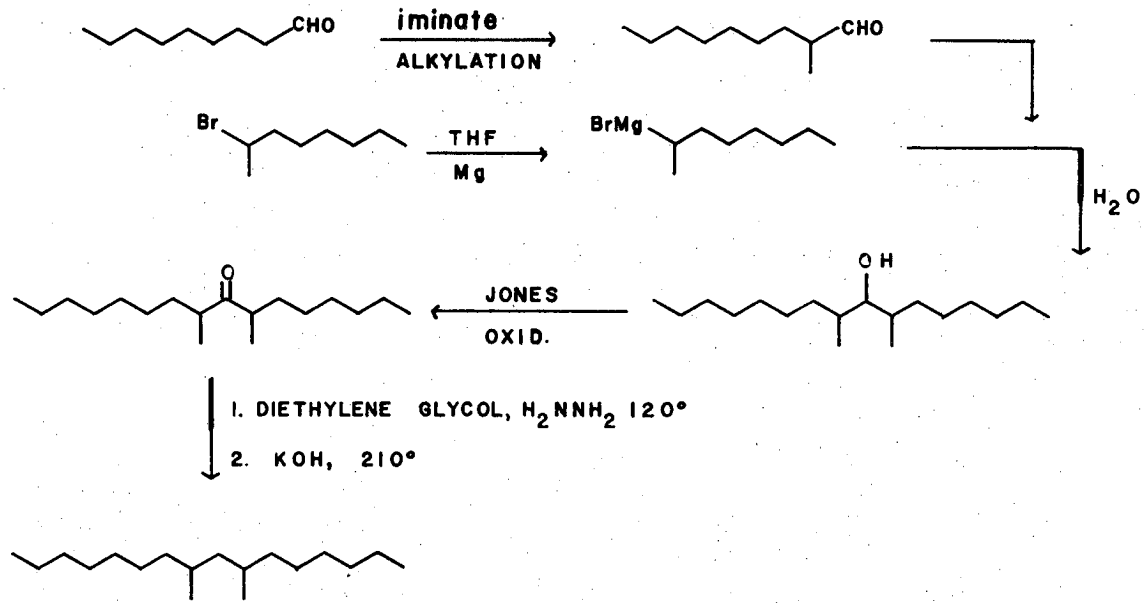
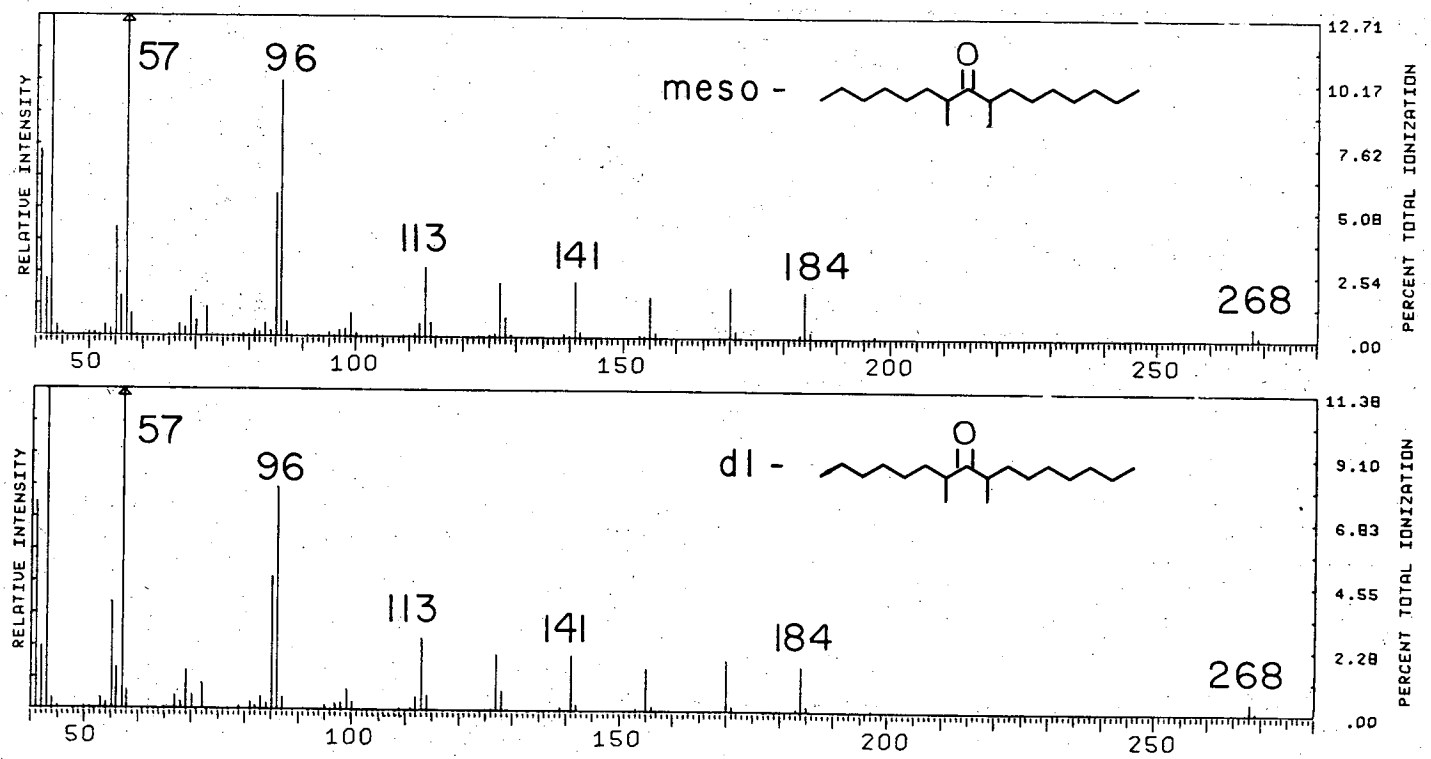


FIGURE 4

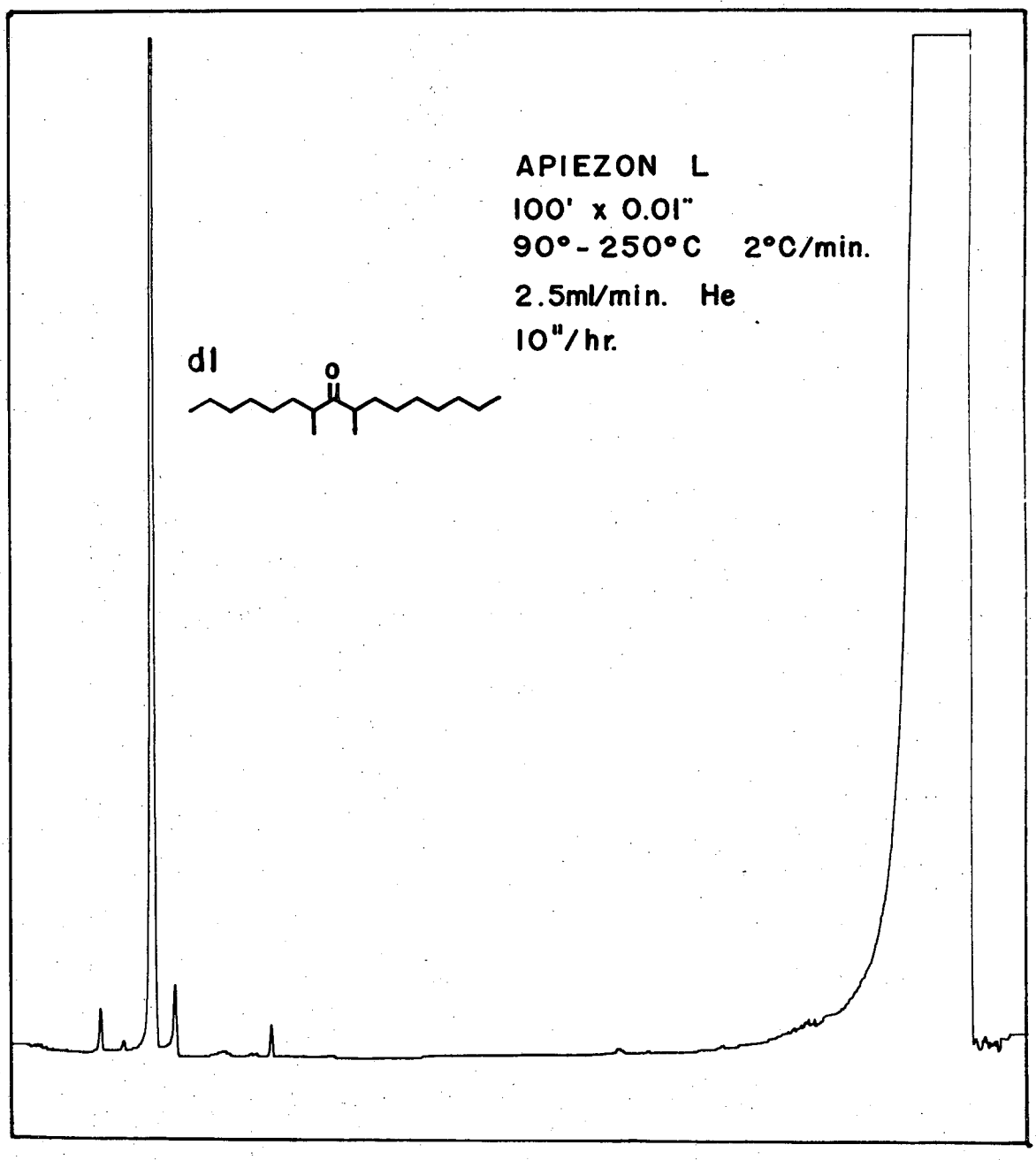
XBL 685-4201

Figure 5: Mass Spectra of the synthetic meso- and dl-7,9-dimethylhexa-8-one obtained using the MS-12. The compounds are ionized by electron impact at 70 ev.


meso -
dl -
57
96
113
141
184
268
RELATIVE INTENSITY
PERCENT TOTAL IONIZATION
12.71
10.17
7.62
5.08
2.54
.00
11.38
9.10
6.83
4.55
2.28
50
100
150
200
250

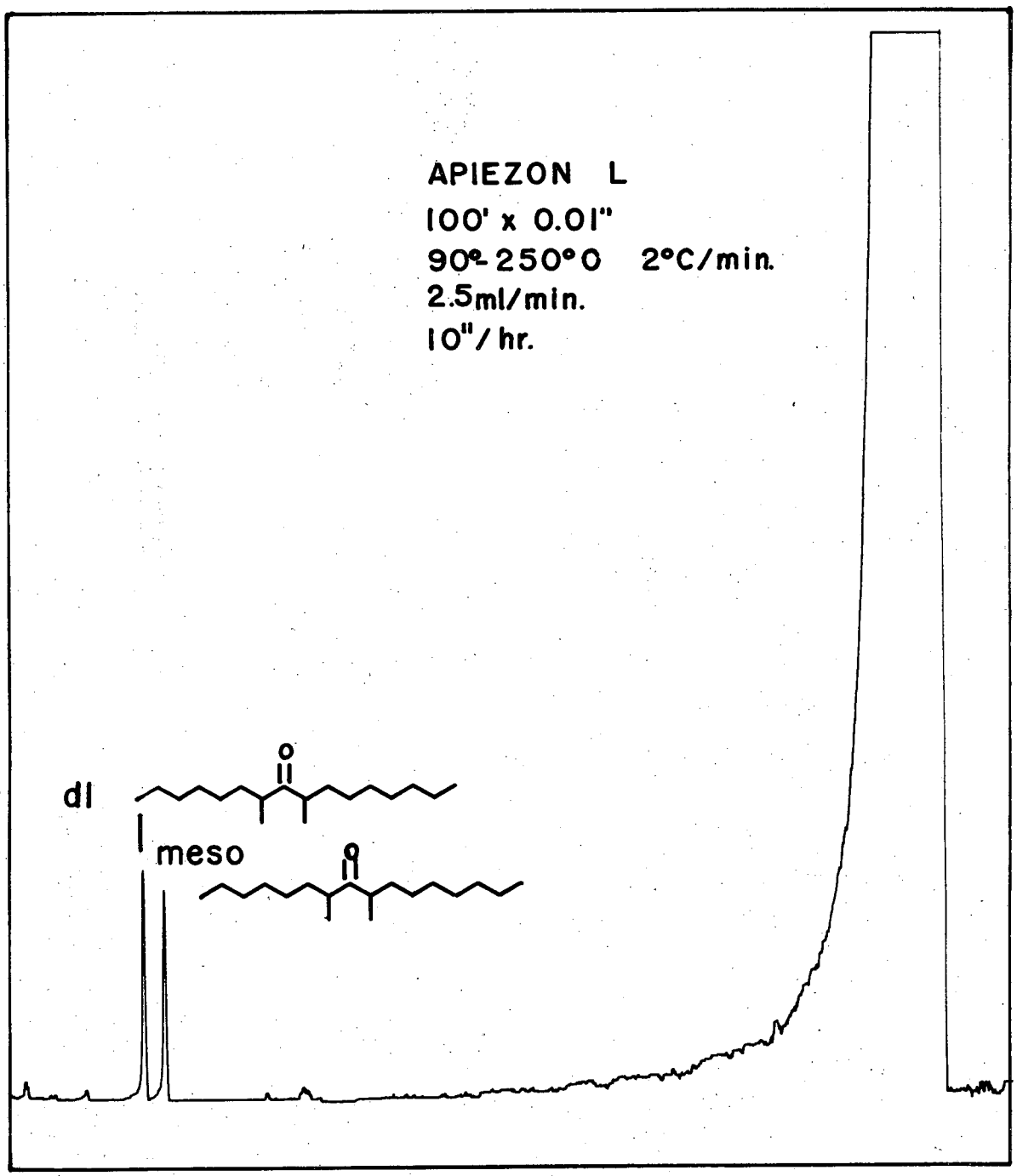
XBL 692-4110

Figure 6: Gas chromatographic separation of synthetic dl 7,9-dimethyl-8-hexadecanone. A stainless steel tubing 100 ft. x 0.01 in. coated with Apiezon L. Aerograph model 204 gas chromatograph was equipped with a flame ionization dector. Helium pressure, 20 psi. No split. Temperature was programmed at 2°C/min. from 90°C to 250°C.



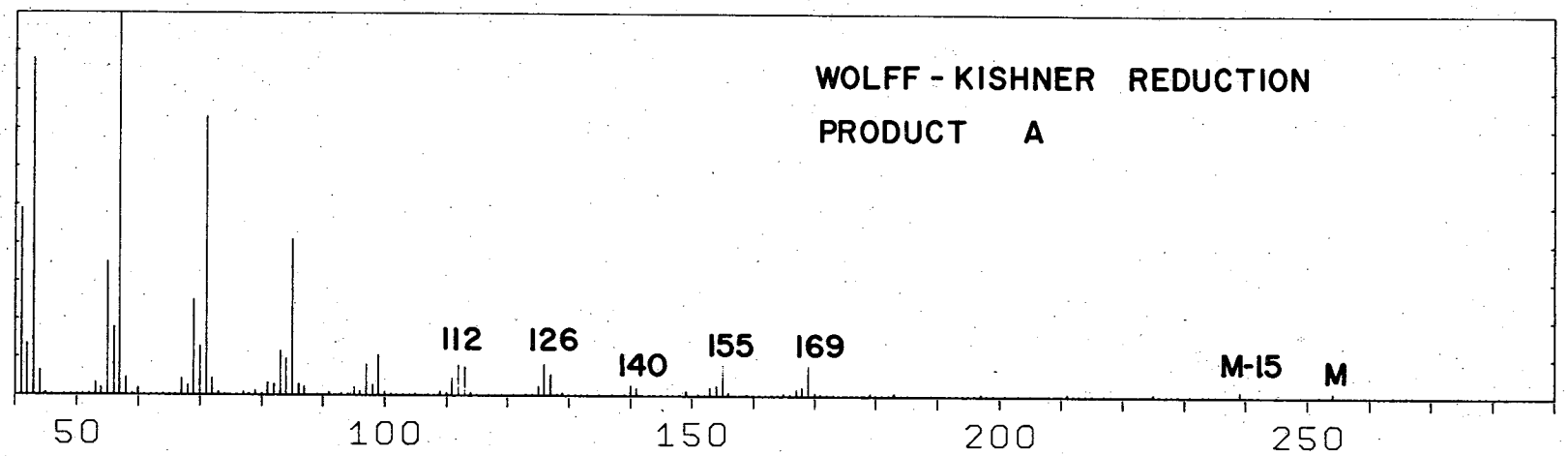
XBL 683-4089

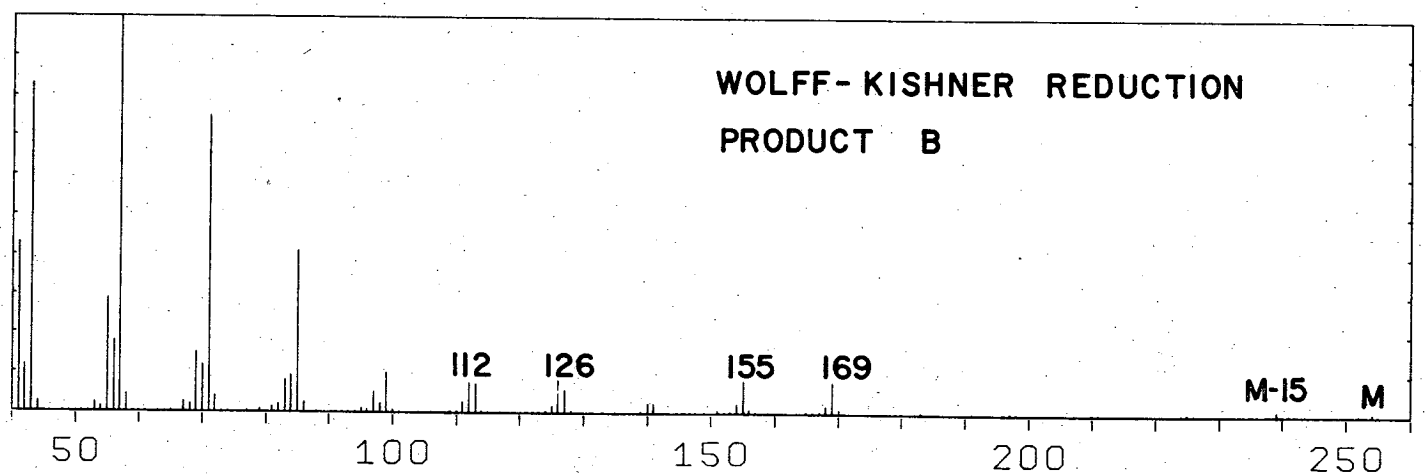
Figure 7: Gas chromatographic separation of epimerized products of dl 7,9-dimethyl-8-hexadecanone. All conditions are the same as Figure 6.



XBL 683-4088

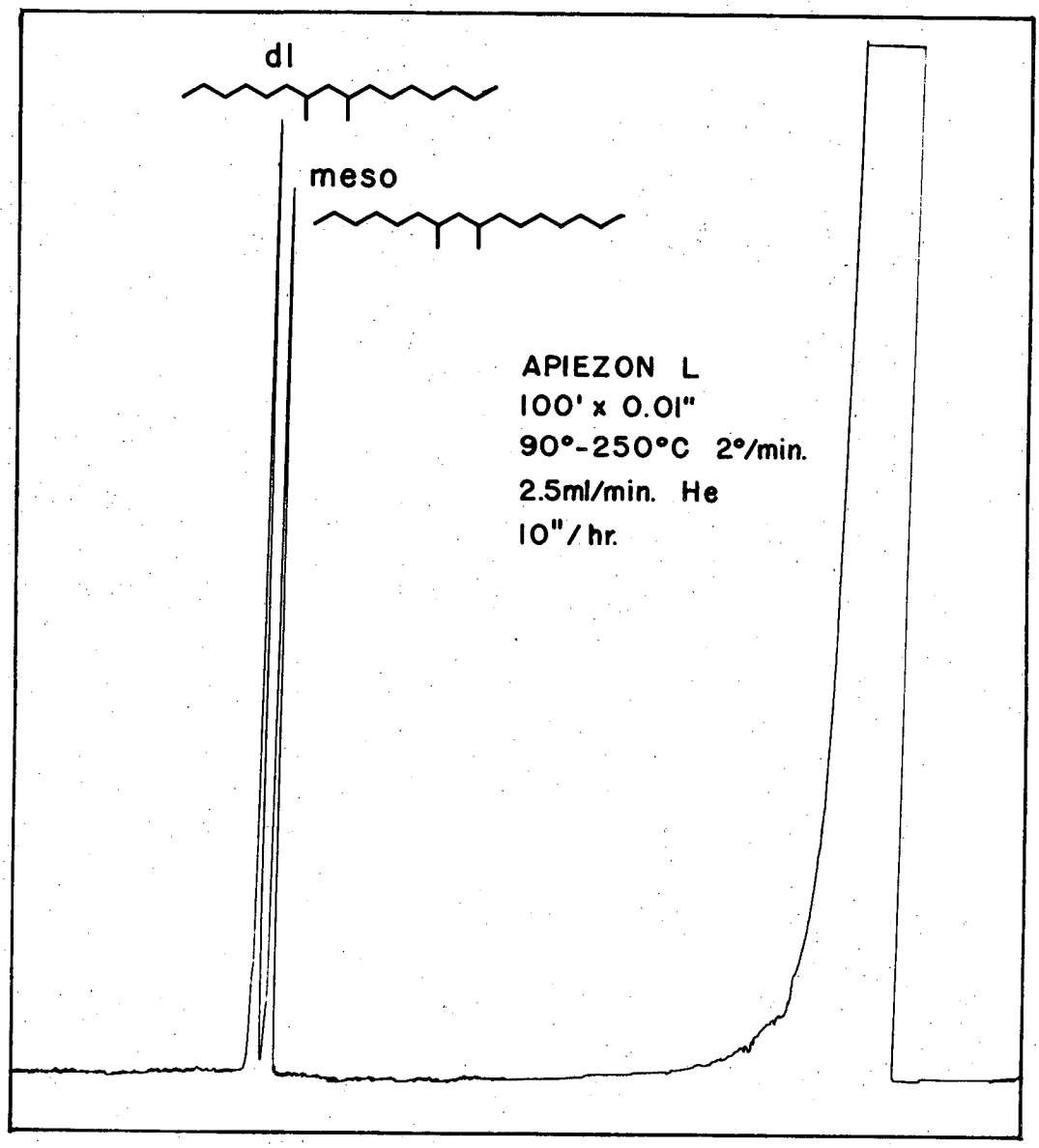
Figure 8: Mass spectra of synthetic 7,9-dimethylhexadecane. All the conditions are the same as Figure 5. (A.) dl isomer. (B.) meso isomer.


WOLFF - KISHNER REDUCTION
PRODUCT A
112
126
140
155
169
M-15
M
50
100
150
200
250


WOLFF- KISHNER REDUCTION
PRODUCT B
112
126
155
169
M-15
M
50
100
150
200
250

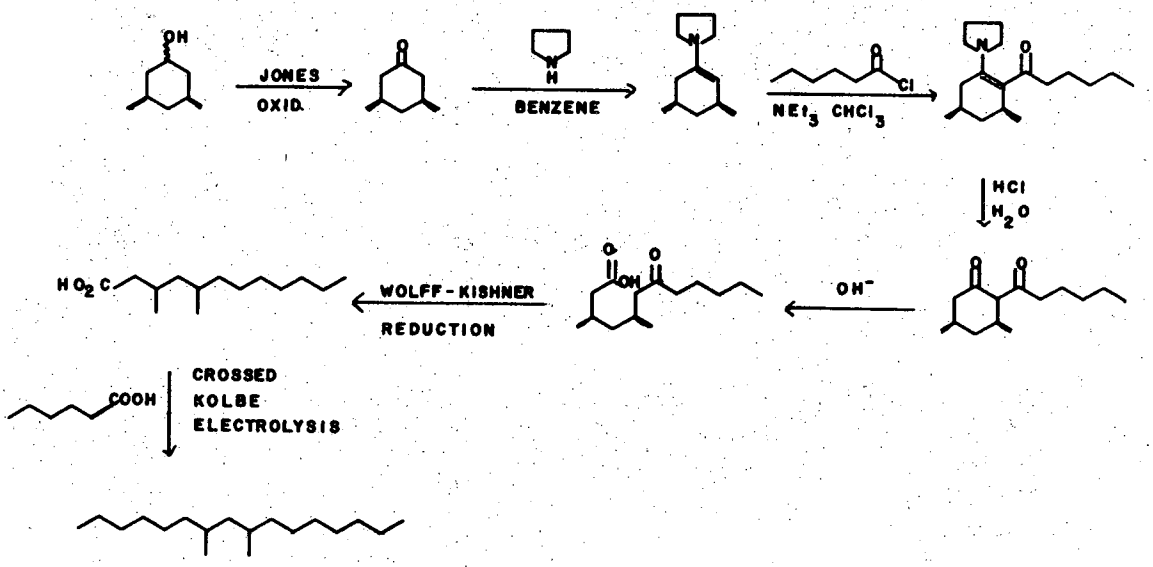
XBL 683-4105

Figure 9: Gas chromatographic separation of non-stereospecific synthesis product of 7,9-dimethylhexadecane. All conditions are the same as Figure 6.



XBL 683-4090

Figure 10: Stereospecific synthesis of meso-7,9-dimethylhexadecane.


OH
JONES
OXID.
BENZENE
NEt3 CHCl3
HCl
H2O
OH⁻
WOLFF-KISHNER
REDUCTION
HO2C
COOH
CROSSED
KOLBE
ELECTROLYSIS

XBL 685-4203

The mass spectra of the mixture of diastereoisomers and of the pure erythro diastereoisomers were identical (Fig. 8). However, they differed significantly enough from the mass spectrum of the C_{18} branched alkane from Nostoc in the relative intensities of the m/e 154, 155 and m/e 168, 169 peaks to induce doubt about its identity; the odd peaks were now more intense than the even peaks. Furthermore, the retention time of the C_{18} branched alkane from Nostoc was greater than the retention times of either of the diastereoisomer standards which had been synthesized. This last fact convinced us that the structure of the Nostoc C_{18} branched alkane was not 7,9-dimethylhexadecane.

The only other feasible explanation was an equal mixture of the 7-methyl- and 8-methyl heptadecanes, which could not be separated by capillary gas chromatography. Such a mixture would be expected to give an almost identical mass spectrum to that of the Nostoc C_{18} branched alkane and in addition should have retention times closer to that of this hydrocarbon. Therefore, one important question remained: Were these two hydrocarbons inseparable by our capillary gas chromatography? Such a question could only be resolved by synthesis of the 7-methyl and 8-methyl heptadecanes. The synthetic procedure used for these two compounds is given in Figure 11. Methyl heptadecanes were synthesized from methyl ketones and 1-bromoalkanes. Grignard reaction was applied to the synthesis of two tertiary alcohols, 7-methylheptadeca-7-ol and 8-methylheptadecan-8-ol. Their spectra were shown in Fig. 12, no molecular ion could be found. In each case after dehydration reactions 7-methylheptadecene and 8-methylheptadecene and five alkene isomers were separated by capillary gas chromatography. The final products 7-methylheptane and 8-methylheptane were obtained by hydrogenation of the alkenes. Fig. 13 shows

Figure 11: Synthetic routes to the 7-methyl-heptadecane and 8-methyl-heptadecane.

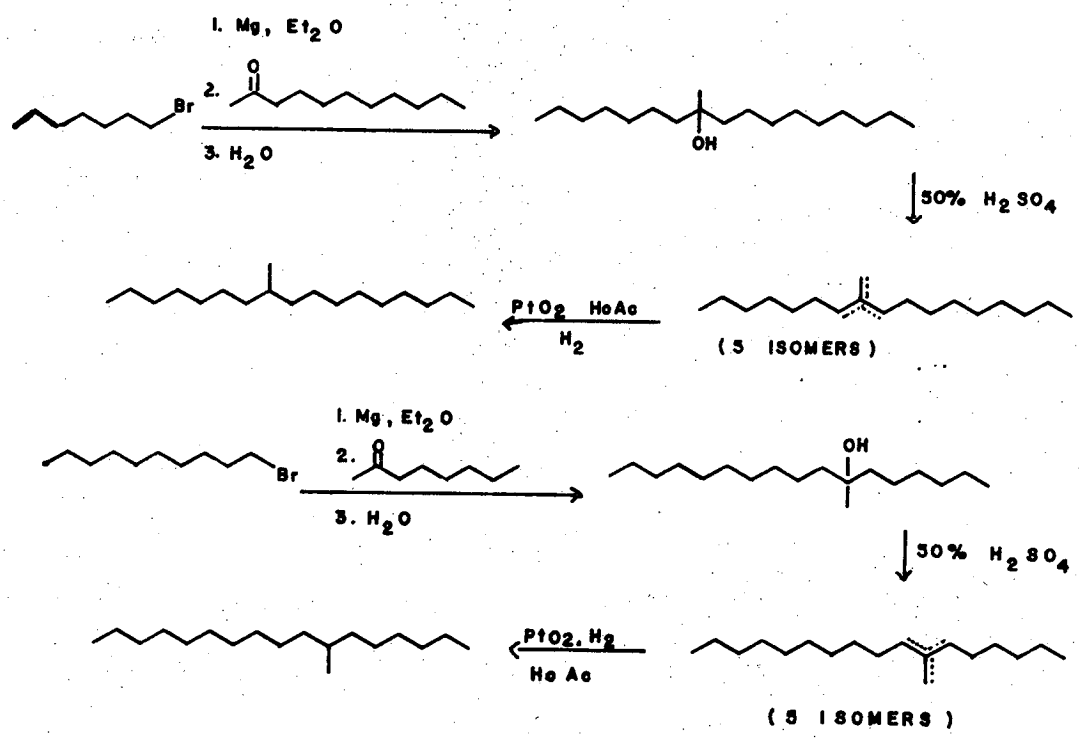
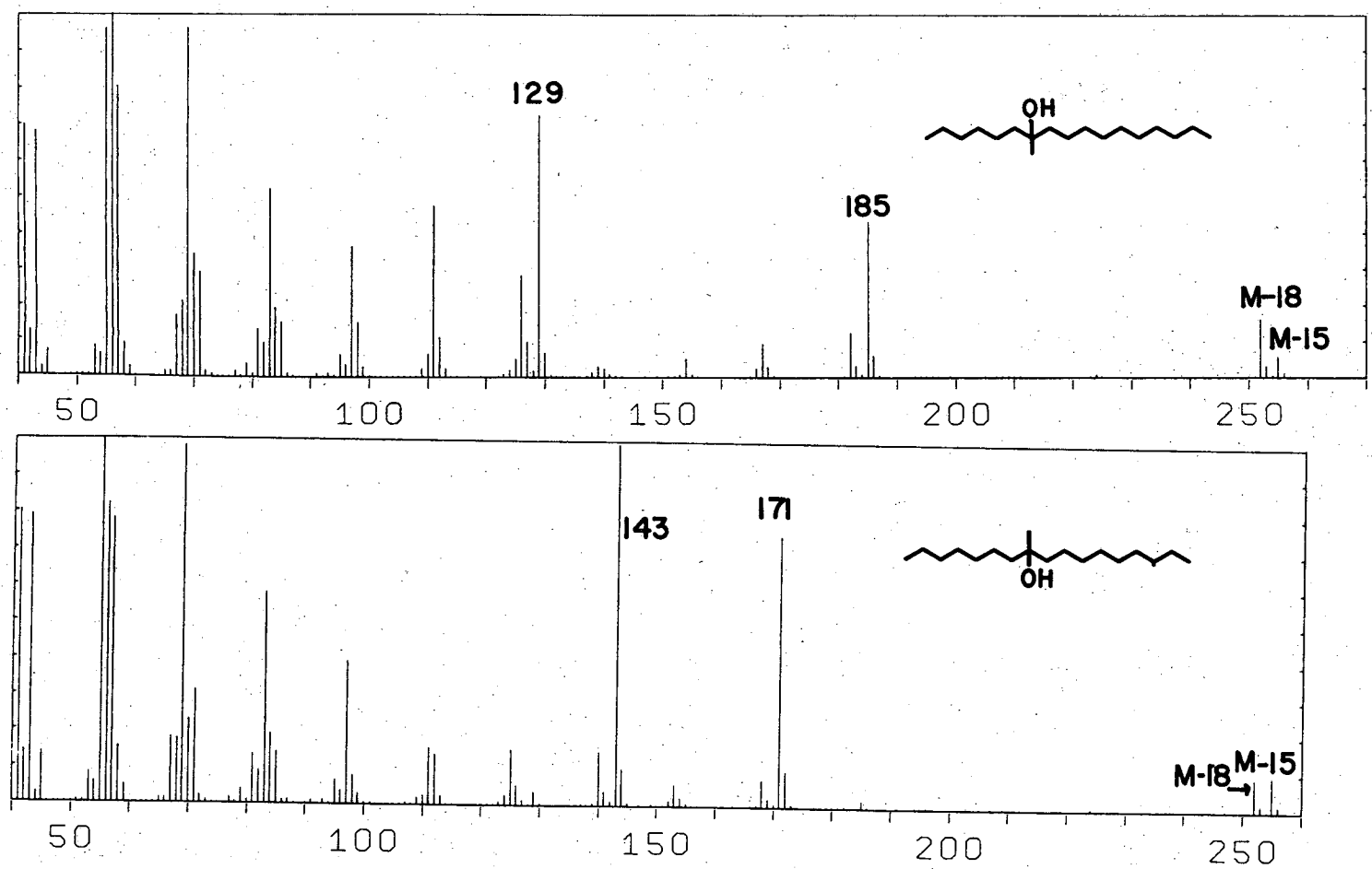


FIGURE 6

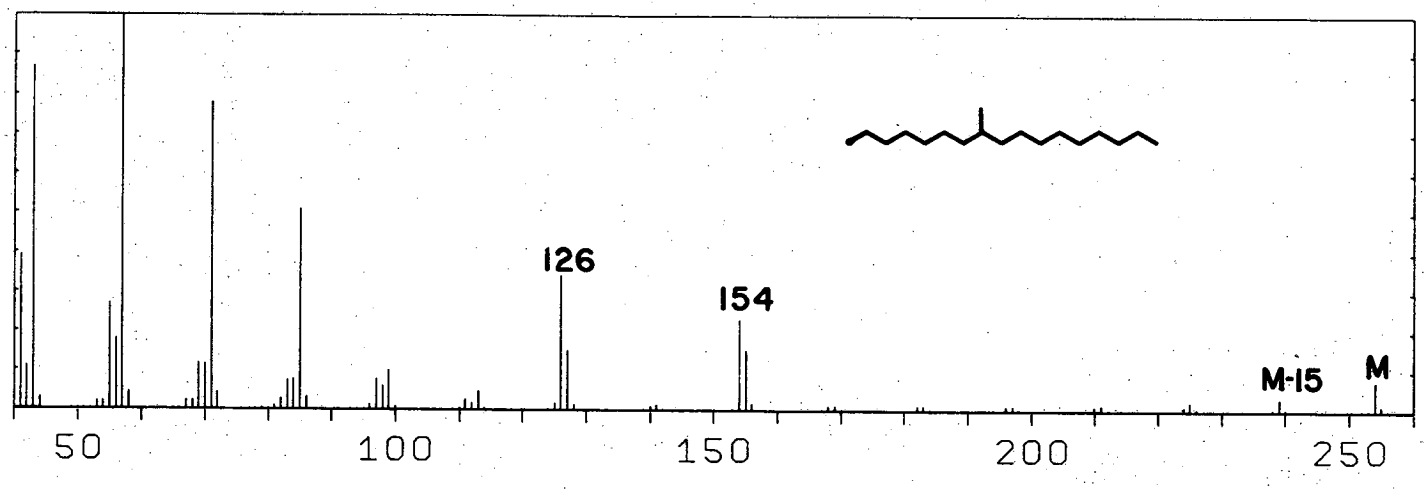
XBL 685-4202

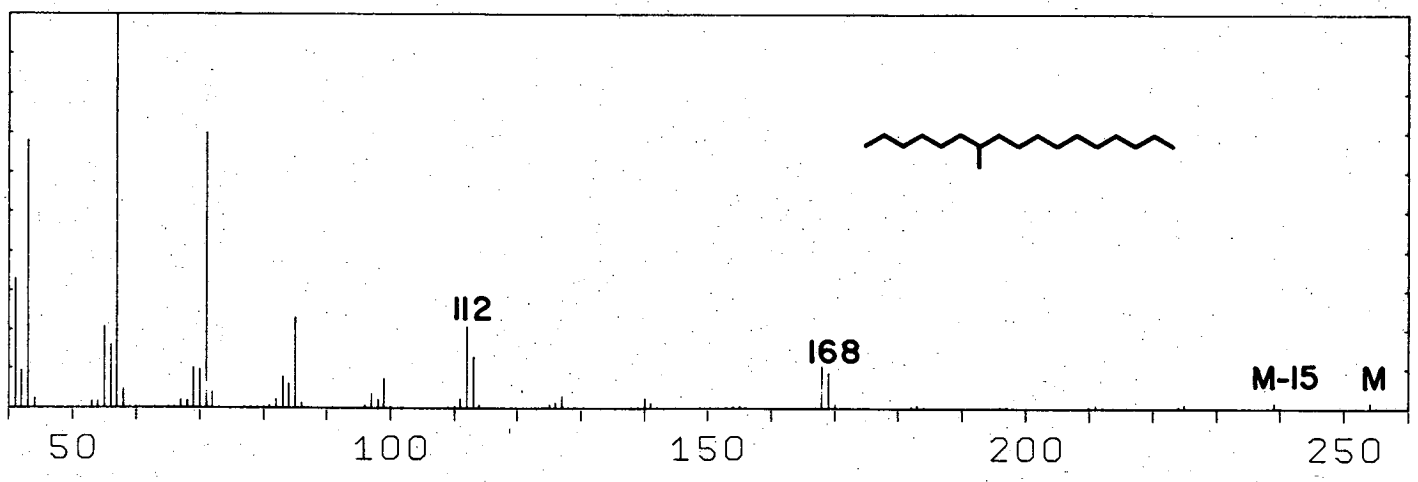
Figure 12: Mass spectra of synthetic 7-methyl-7-heptadecanol and 8-methyl-8-heptadecanol. All the conditions are the same as Figure 5.


129
185
OH
M-18
M-15
50
100
150
200
250
143
171
OH
M-18
M-15
50
100
150
200
250

XBL 683-4106

Figure 13: Mass spectra of synthetic 7-methylheptadecane and 8-methylheptadecane. All the conditions are the same as Figure 5.

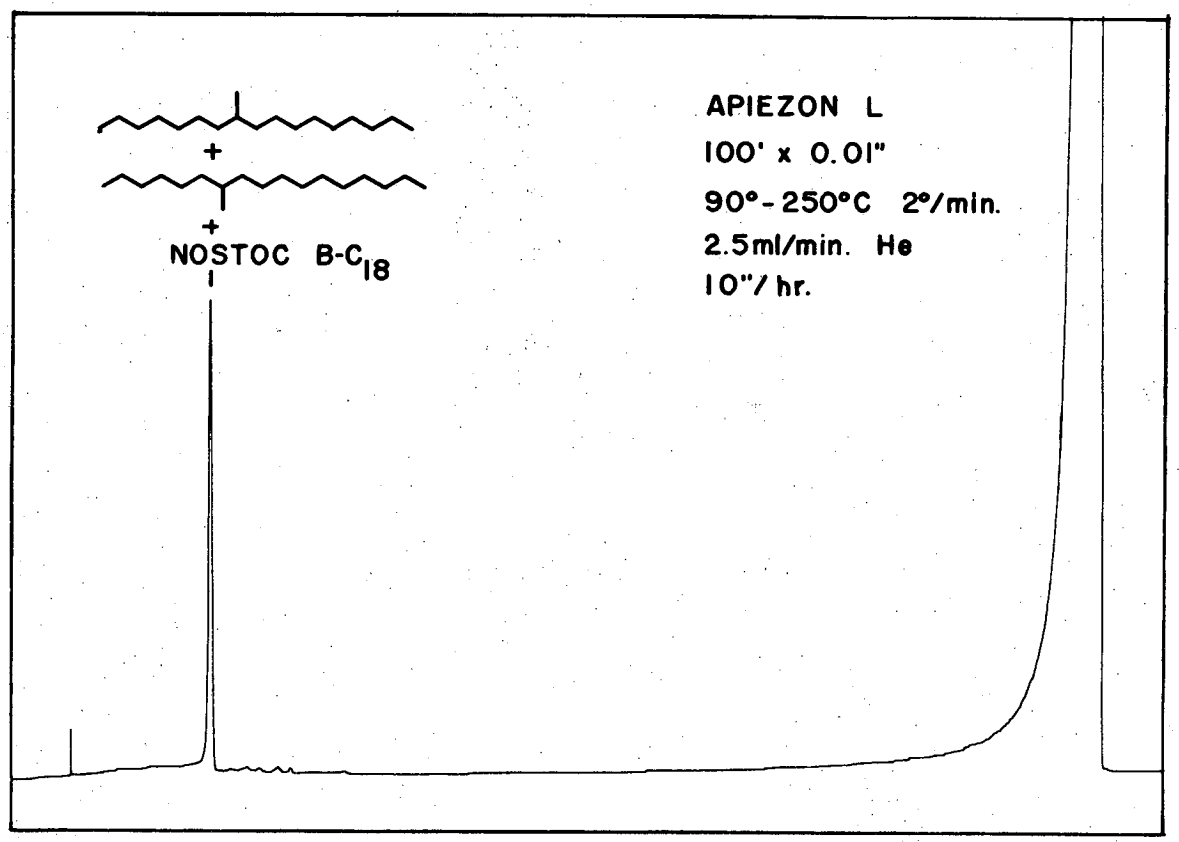




XBL 683-4107

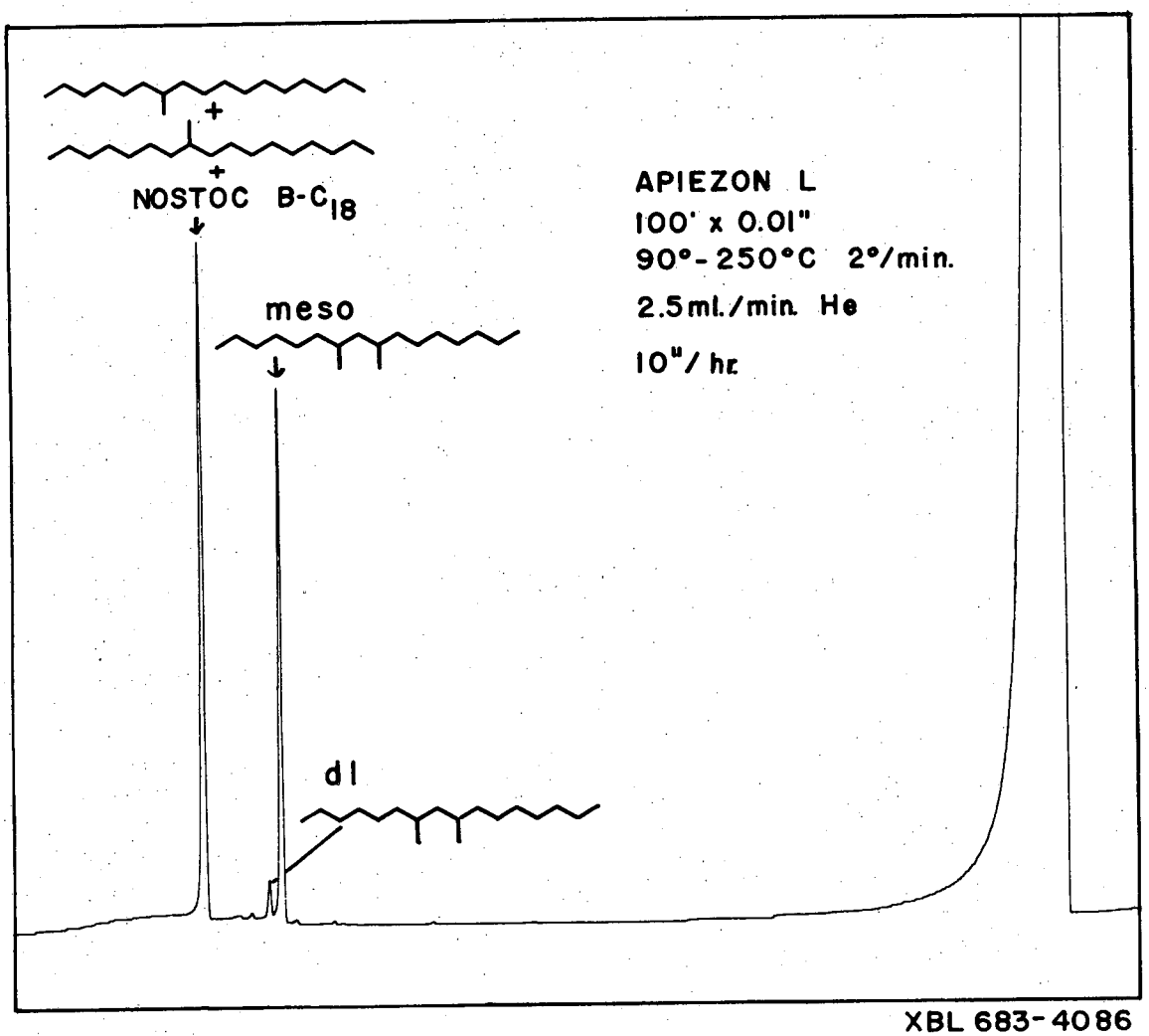
their mass spectral characteristics, the peaks, 126 and 154 in one spectrum and 112 and 168 in the other, were now more intense than the odd ones. Neither the 7-methyl nor 8-methylheptadecanes, nor the C_{18} branched alkane from the Nostoc could be separated by capillary gas chromatography (Fig. 14). Fig. 15 shows the gas chromatographic separation of all these dimethylhexadecane isomers and methylheptadecane isomers.

Figure 14: Gas chromatographic coinjection of 7- and 8-methylheptadecane and branched-C_{18} from Nostoc is obtained with an Aerograph-204 instrument.



XBL 683-4087

Figure 15: Gas chromatographic separation of branched-C_{18} hydrocarbon and synthetic dl and meso-7,9-dimethylhexadecane, 7-methylheptadecane and 8-methylheptadecane.


NOSTOC B-C18
meso
dl
APIEZON L
100' x 0.01"
90°-250°C 2°/min.
2.5ml./min. He
10"/hr.

XBL 683-4086

EXPERIMENTAL SECTION

2-Methylnonanal[4]

Nonanal[5] (6.8g) was added to a solution of t-butyl amine (18g) and p-toluenesulphonic acid (ca. 100mg) in petroleum ether (b.p. 40°-60°, 150ml), and the reaction mixture was boiled for 6 hrs. under a Dean-Stark water-separating reflux-head. The solution was decanted from a little brown oil and evaporated to a pale greenish-yellow oil which was fractionally distilled to yield nonanal t-butylimine b.p. 82°/1mm (3.8g). νmax 1660, 1201cm^{-1}.

The t-butylimine (3.8g) in dry tetrahydrofuran (10ml) was added to a Grignard reagent prepared from ethyl bromide (2.18g) and magnesium (0.460g) in dry tetrahydrofuran (30ml), and the reaction mixture was boiled under a reflux condenser for 24 hrs, with exclusion of atmospheric moisture. Methyl bromide was added dropwise before aqueous hydrochloric acid (6N, 200ml) was added and the mixture boiled for a further 2 hrs, then cooled and extracted with ether (100ml). The ether extract was washed with water (1 x 100ml), and the aqueous wash re-extracted with ether (100ml). The combined ether extracts were dried ($MgSO_4$) and evaporated to a residual oil which was fractionally distilled to yield 2-methylnonanal, b.p. 52°/0.4mm (0.77g); homogenous by GLC analysis (< 3% of more volatile contaminant, apparently nonanal) ν max 2680, 1715cm^{-1}.

7,9-Dimethyl-8-hexadecanone

2-methylnonanal (0.77g) was added to a Grignard solution, obtained from 2-bromooctane (1.10g) and magnesium turnings (0.140g) in dry tetrahydrofuran (10ml), and boiled gently for 6 hrs, then kept overnight at

room temperature. Saturated aqueous ammonium chloride solution (0.6ml) was added to the reaction mixture which was stirred for a further 10 min., then filtered through a celite pad. The filter pad was washed with ether, and the filtrate and washings were evaporated to yield 7,9-dimethyl-8-hexadecanol as a yellowish oil.

The hexadecanol was dissolved in acetone (50ml), the solution cooled to 0°, and titrated with Jones' reagent[6] (2.8ml required). The acetone solution was decanted from inorganic salts, which were washed with ether (50ml), and the supernatant and washings were evaporated to a small volume which was then partitioned between water (20ml) and ether (20ml). The aqueous phase was reextracted with ether (20ml) and the combined ether extracts were dried ($MgSO_4$), evaporated and the residue fractionally distilled to yield 7,9-dimethyl-8-hexadecanone, b.p. $120\text{-}125^{o}/0.3$ mm. (0.50g).

The 7,9-dimethyl-8-hexadecanone was separated into two equal peaks on a 25' x 0.25" 3% SE-30 column. 10mg of each of these two components were isolated by preparative gas chromatography, Aerograph Model A-90P. They had identical infrared spectra and mass spectra. νmax=1374, 1707cm^{-1}, m/e=268 (molecular ion), 184 $(C_{12}H_{24}O)^+$ McLafferty rearranged fragment, 170 $(C_{11}H_{22}O)^+$ McLafferty rearranged fragment, 155 $(C_{11}H_{23})^+$, 141 $(C_{10}H_{21})^+$, 127 $(C_8H_{19})^+$, 113 $(C_8H_{17})^+$.

<u>Epimerization of 7,9-dimethyl-8-hexadecanone[7]</u>

5.5 grams of potassium hydroxide in 100ml ethanol was prepared in a 250ml round-bottomed flask. After 6mg of the first 7,9-dimethyl-8-hexadecanone (the one with shorter retention time on preparative GLC)

had been added, the stirred mixture was heated under reflux for two hours. The solution was cooled to room temperature and was acidified with concentrated sulfuric acid. The potassium sulfate salt was filtered out and washed with 10ml of ethanol. Most of the solvent was removed on a rotary evaporator. The crude product was taken up in 0.5ml of ethanol and quantitatively transferred to the top of a 100g activated aluminum oxide column. The column was then successively eluted with 80ml each of n-heptane and diethyl ether. The ether eluate was evaporated to dryness and yielded 4.8 mg. of ketones. These products were checked by GLC again and contained a 50:50 mixture of the first and second ketones. Both show m/e 268 (molecular ion, 184, 170 (McLafferty rearranged fragments), 155, 141, 127 and 113 on mass spectra.

The second ketone (6mg) was treated in an identical manner and yielded 5.1 mg of a 50:50 mixture of the first and second ketones.

Wolff-Kishner Reduction of 7,9-dimethyl-8-decanone[8,9].

A mixture of erythro and threo ketones(6mg,)hydrazine hydrate(0.05ml,) and freshly distilled diethylene glycol(0.20ml)was placed in an 18cm x 0.8cm Pyrex tube. The tube was heated in an oil bath at 120°C for 2 1/2 hrs. After cooling, powdered KOH (0.2g) was added. The tube was cooled with liquid nitrogen and sealed under 5 mm. Hg vacuum. The tube was put back into the oil bath, the temperature was slowly raised to 210°C, and maintained at 210°C for 2 1/2 hrs. After cooling to room temperature, the reaction mixture was poured into 10ml H_2O and extracted with ether (2 x 5ml). After the solvent was removed, the alkanes were separated by column chromatography.

The n-heptane eluate from the aluminum oxide column yielded a mixture of two 7,9-dimethylhexadecanes, which were separated by GLC on a 100' x 0.01", Apiezon L column at a helium flow rate of 2.5ml per minute, using an Aerograph Model 204 gas chromatograph equipped with a flame ionization detector. The mass spectra of these two 7,9-dimethylhexadecanes were taken by combined gas-chromatographic-mass spectrometric analysis[10,11], which was performed with an Aerograph 204 gas chromatograph combined directly with an A.E.I. MS-12 mass spectrometer and the same capillary column and conditions as described above. These two alkanes had identical spectra; m/e 254 (molecular ion), 239 (M-15), 169 $(C_{12}H_{25})^+$, 155 $(C_{11}H_{23})^+$, 126 $(C_9H_{18})^+$, 112 $(C_8H_{16})^+$.

3,5-dimethylcyclohexanone.

Commercial 2,5-dimethylcyclohexanol (cis-by GLC) was oxidized with Jones' reagent to the ketone b.p. 68-70°/15mm.

3,5-dimethylcyclohexanone pyrrolidine enamine. (1-N-pyrrolidyl-3,5-dimethylcyclohexene.

3,5-dimethylcyclohexanone (23g) was dissolved in benzene (300ml) containing pyrrolidine (20g) and the solution was boiled under a Dean-Stark distill-head for 4 hrs. and then evaporated, under water-pump vacuum, to a residual oil which was fractionally distilled to yield the enamine b.p. 102°/1.5 min. (30.5g). Found C, 80.23; H, 11.90, N, 7.94%. Calculated for $C_{12}H_{21}N$ (179.30), C, 80.38; H, 11.81; N, 7.81%.

2-(2'-ketoheptyl)-3,5-dimethylcyclohexanone[12].

The enamine (17.9g) and triethylamine (12g) were dissolved in dry chloroform (15ml) and hexanoyl chloride (13.5g), dissolved in dry chloroform

(100ml), was added dropwise, with stirring, over 40 min. The reaction mixture was kept 1 hr. at 35° and then kept overnight at room temperature. Aqueous hydrochloric acid (6N, 120ml) was added and the reaction mixture was stirred vigorously and boiled for 5 hr, then poured into water (300ml), the chloroform layer extracted washed with water (3 x 100ml). The individual aqueous washes were reextracted with chloroform (1 x 100ml) and the combined chloroform extracts were dried ($MgSO_4$), evaporated, and the residue fractionally distilled to yield the diketone, b.p. 114-117°/0.9mm. (11.3g) which crystallized on storing at 0°.

3,5-dimethyldodecanoic acid[12]

The dione (8.2g) was heated to 110°, stirred, and a solution of potassium hydroxide (5.0g) in water (5ml), preheated to 110°, was added in one portion. After 4 mins. the solution was poured into water (120ml); the cold solution was acidified with conc. hydrochloric acid and extracted with ether (2 x 50ml). The combined ether extracts were dried ($MgSO_4$) and evaporated to an oily residue which was taken up in diethylene glycol (40ml). Potassium hydroxide (2.1g) and hydrazine hydrate (15ml) were added to this solution and the mixture was boiled under reflux for 8 hrs. A hot solution of potassium hydroxide (9.0g) in diethylene glycol was then added, the bath temperature raised, and the excess hydrazine allowed to boil away until the solution temperature had reached 200°. The solution was then boiled under reflux for 14 hrs., after which it was cooled, diluted with water (800ml), acidified with concentrated hydrochloric acid and extracted with ether (2 x 100ml), then dried ($MgSO_4$), evaporated and the residue fractionally distilled to yield the acid, b.p. 134-135°/0.8 mm (2.90g). Found C, 74.02; H, 12.48%. Calculated for $C_{14}H_{28}O_2$ (228.36), C, 73.67; H, 12.36%.

7,9-Dimethylhexadecane.

3,5-Dimethyldodecanoic acid (2.90g) and hexanoic acid (5.75g) were dissolved in methanol (200ml) in which sodium (1.0g) had been dissolved. The solution was electrolyzed[13] for 2 hrs., by which time the cell current had fallen from the initial value of 4 amp. to a steady value of 0.4 amp. Some waxy solid separated during the electrolysis. The solvent was distilled off at atmospheric pressure, through a 25 cm. Vigreux column until only a small volume of solution remained. This was taken up in ether (2 x 200ml), previously used to rinse the cell and electrodes, the volume again reduced to about 100ml. and the solution was then washed with aqueous sodium hydroxide (ca.1N, 50ml). The ethereal solution was dried ($MgSO_4$), evaporated and the residue fractionally distilled to yield the 7,9-dimethylhexadecane as a fraction b.p. 162°/15mm. (0.76g). GLC analysis revealed this to consist of a "single" major component contaminated with less than 5% of other, more volatile material.

7-methylheptadecane-7-ol

1-bromodecane (10g) in dry ethyl ether (15ml) was added dropwise to a 150ml three necked flask with magnesium (1g) in dry ether (15ml)[14]. The bromide solution was added over one hour after which the mixture was heated under reflux for two hours with exclusion of air. The reaction mixture was cooled to $-8^{o}C$ in an ice and salt bath. A solution of 6g of freshly prepared 2-octanone in 15ml of anhydrous ether was added dropwise to the reaction mixture, the temperature remaining between $-8^{o}C$ and $0^{o}C$. After all the ketone had been added, the solution was stirred for 5 mins. and

then poured onto 30g of cracked ice in a 250ml beaker. The mixture was acidified with 10% sulfuric acid and the aqueous layer was separated and extracted with 10ml ether. The solvent was removed on a rotary evaporator to yield 7-methyl-heptadecan-7-ol, (12g). νmax=1145, 1374, 3380cm^{-1}; m/e 252 (M-18), 255 (M-15), 129 $(C_8H_{16}OH)^+$, 185 $(C_{12}H_{24}OH)^+$.

7-methylheptadecene

The 7-methylheptadecan-7-ol (6g) in 50 ml of n-hexane was added to 50 ml 50% sulfuric acid. The mixture was stirred for 5 hrs. The organic layer was separated and the aqueous solution was washed with 10ml n-hexane. The combined n-hexane solution was concentrated to yield 7-methylheptadecane (5.5g) νmax=890, 1650, 1374cm^{-1}, m/e 252 (molecular ion).

7-methylheptadecane

A mixture of 7-methylheptadecene (2.75g), isopropyl alcohol (20ml), glacial acetic acid (2ml) and PtO_2 (5mg) were placed in a Brown Hydrogenator [15,16]. Hydrogen was generated from acetic acid and sodium borohydride. After the reaction solution was stirred for five hours, most of the solvent was removed on a rotary evaporator. The crude product was transferred to the top of an activated aluminum oxide (100g) column. The column was then eluted with 100ml of n-heptane. The n-heptane fraction yielded 2.5g of 7-methylheptadecane. m/e 254 (molecular ion), 168 $(C_{12}H_{24})^+$, 112 $(C_6H_{16})^+$, νmax=1374cm-1.

8-methylheptadecan-8-ol

An identical procedure as with 7-methylheptadecan-7-ol was used. Starting material: 1g magnesium, 8g 1-bromoheptane and 7g 2-undecanone. Final product: 8-methylheptadecan-8-ol (11g), νmax=1145, 1374, 3380cm^{-1}; m/e 252 (M-18), 255 (M-15), 143 $(C_9H_{18}OH)^+$, 171 $(C_{11}H_{22}OH)^+$.

8-methylheptadecene

The 8-methylheptan-8-01 (6g) was dehydrated with 50% sulfuric acid (see procedure for 7-methylheptadecene) yielding 8-methylheptadecene (5.5g). νmax=890, 1650, 1374cm^{-1}, m/e 254 (molecular ion).

8-methylheptadecane

The 8-methylheptadecene (2.75g) was hydrogenated in a Brown Hydrogenator[33,34] (same procedure was used as in 7-methylheptadecane). Final product 8-methylheptadecane (2.7g). νmax=1374cm^{-1}, m/e 254 (molecular ion), 154 $(C_{11}H_{22})^+$, 126 $(C_9H_{18})+$.

4. Final Identification and Physical Measurements

The mass spectrum of a 50:50 mixture of these hydrocarbons corresponded exactly with the mass spectrum of the C_{18} branched alkane from Nostoc. A comparison of these mass spectra is shown in Figure 16. Finally, a scrutiny of the NMR spectrum of the C_{18} branched alkane from Nostoc showed that it corresponded more closely with the mixture of 7-methyl and 8-methyl heptadecanes rather than with the NMR spectrum of the 7,9 dimethylhexadecane. Therefore, the structure of the C_{18} branched alkane isolated from the blue-green alga, Nostoc, and from the other species, corresponds to an equal molar mixture of 7-methyl and 8-methyl heptadecane.

The optical activity was studied on an automatic polarimeter. 5mg of Nostoc branched-C_{18} hydrocarbon was dissolved in 2.5 ml of hexane and measured with sodium D line. An optical rotation of $+2.5 \pm 0.5$ was obtained. No further attempt has been made to designate the exact stereochemistry or absolute configuration at the asymmetric center.

Finally, high resolution gas chromatography was carried out by using a 750 ft., 0.02 inch inside diameter column coated with 50% Igepal Co-880 and 50% GE SF-96. The total hydrocarbon fraction (2 ug) from Nostoc was injected at 30 Psi of helium as carrier gas and oven temperature isothermally at 125°C. The branched C_{18} hydrocarbon mixture was indeed separated into an equal amount of 7-methylheptadecane (Fig. 17, peak b) and 8-methylheptadecane (Fig. 17, peak a). However, approximately 10% of peak C was also separated by this high resolution gas chromatography. Peak c was identified as 6-methylheptadecane by GC-MS.

Figure 16: Mass spectrum of a 50:50 mixture of synthetic 7- and 8-methyl-heptadecane is obtained with an A.E.I. MS-12 mass spectrometer.

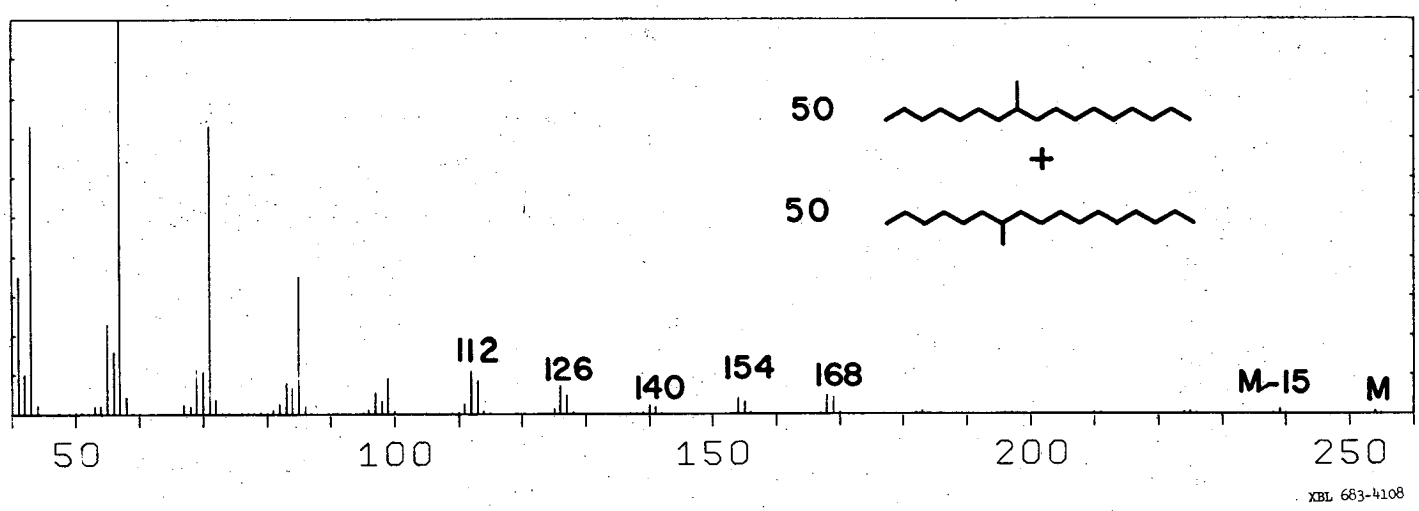
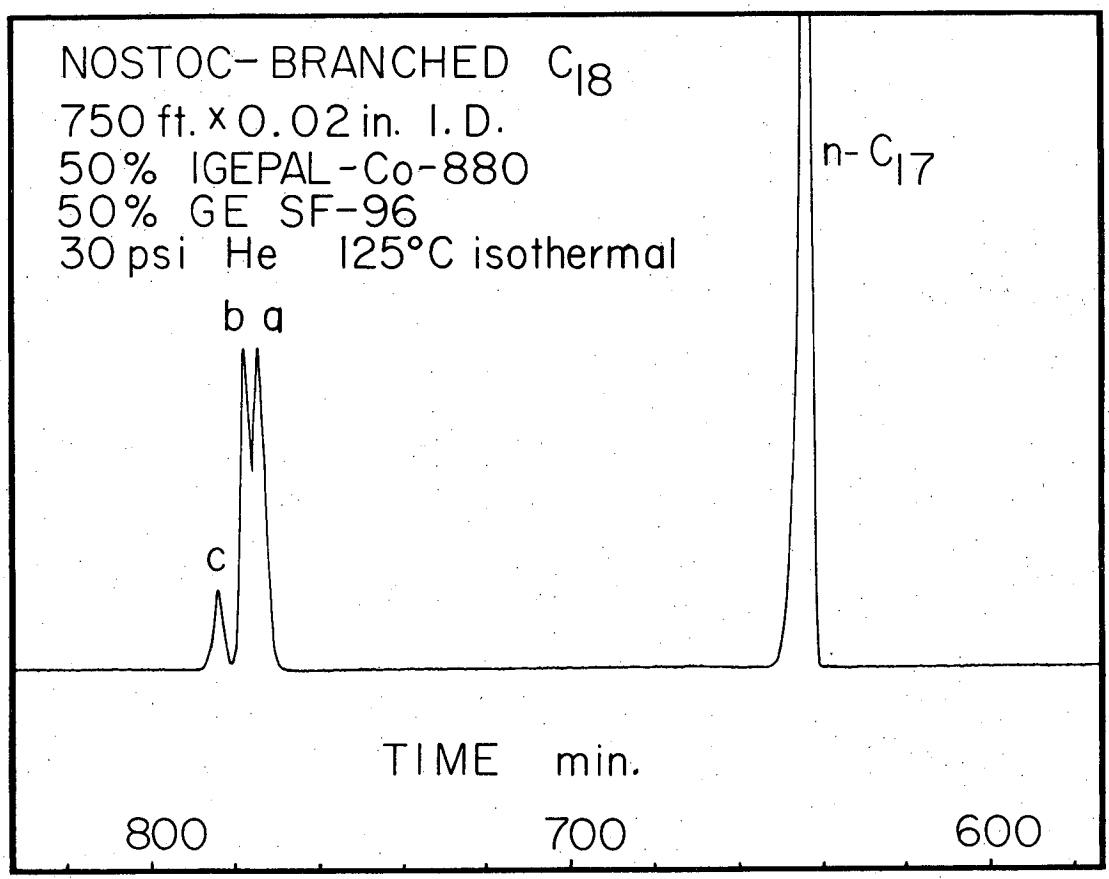

50
+
50
112
126
140
154
168
M-15
M
50
100
150
200
250
XBL 683-4108

Figure 17: Gas chromatographic separation of branched-C_{18} hydrocarbons. A stainless steel tubing 750 ft. x 0.02 in. coated with 50% Igepal-CO-880 and 50% of GE SF-96. Aerograph model 204 gas chromatograph was equipped with a flame ionization detector. Helium pressure 30 psi, no split. Isothermal at 125°C. Peaks a, b, and c were identified as 8-methyheptadecane, 7-methyl-heptadecane, and 6-methylheptadecane, respectively by GC-MS.


NOSTOC-BRANCHED C_{18}
750 ft. x 0.02 in. I.D.
50% IGEPAL-Co-880
50% GE SF-96
30 psi He 125°C isothermal
b a
c
n-C_{17}
TIME min.
800
700
600

XBL 702-5052

5. Geological Significance

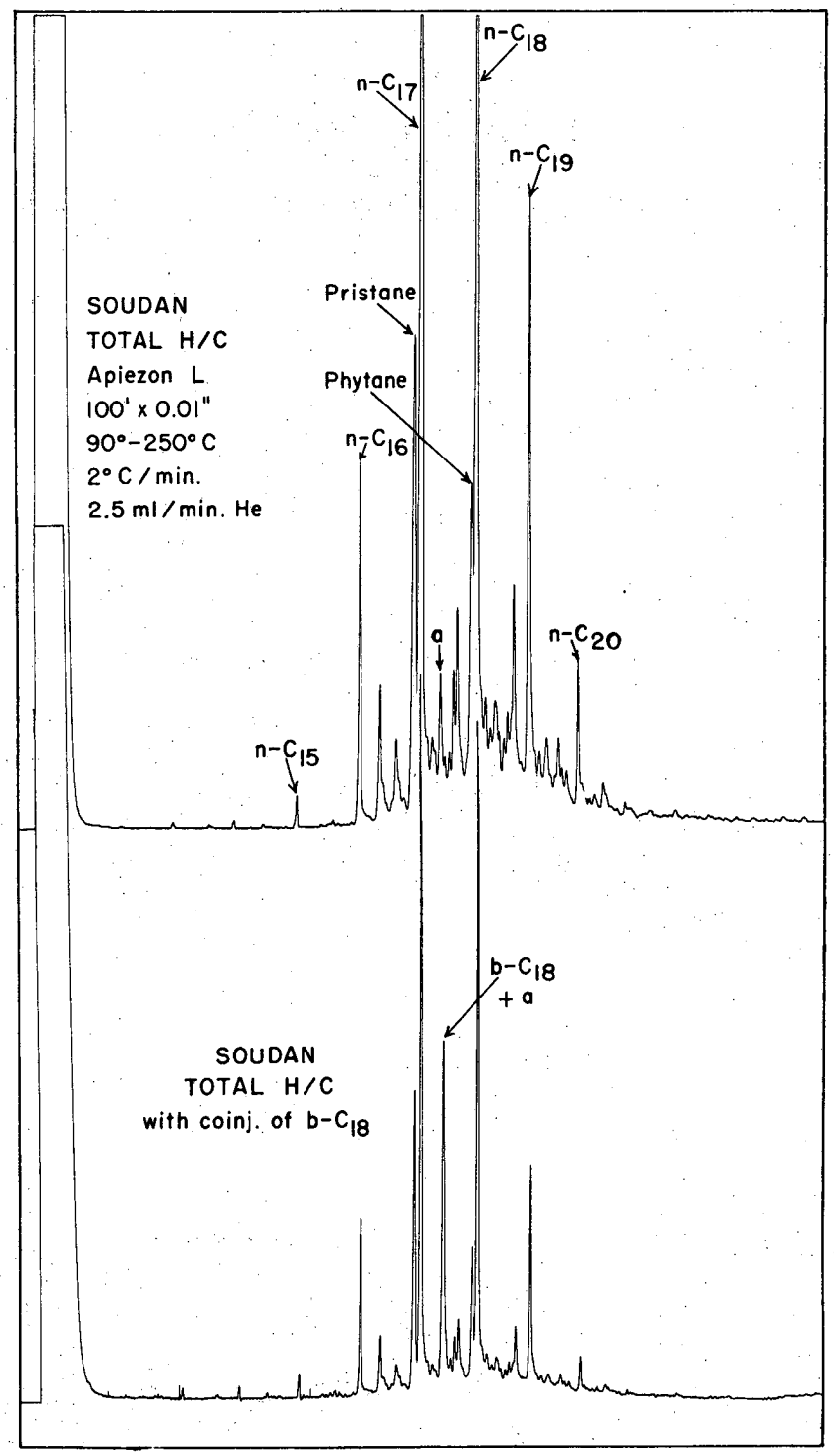
In an organic geochemical context it is always interesting to look for the occurrence of biological markers in Precambrian sediments. Barghoorn and Tyler,[17,18] and more recently Barghoorn and Schopf[19-22], reported the presence of spheroidal microfossils. They were interpreted as probably representing the remnants of unicellular blue-green algae-like and bacterium-like organisms. The presumed photosynthetic nature of these primitive algae seems corroborated by carbon isotopic studies of organic matter in the early Precambrian rocks. The branched C_{18} hydrocarbon becomes an extremely important biological marker to prove the occurrence of primitive algae in that age. Such a finding would elegantly complement the paleobotanical studies of these workers.

Three different columns, 100 ft., x 0.01 in. I.D. HI-EFF-4BP, and 150 ft. x 0.01 in. I.D. SF-96 were used for this analysis. Coinjection of authenticated branched C_{18} mixture to a total hydrocarbon fraction of Soudan Shale (2.7 x 10^9 years) was made on all three columns. The location of the standard coincided with the peak labeled 'a' in Fig. 18. This implies that blue-green algae were probably present at the time of the formation of the Soudan Shale.

6. 4-methylheptadecane

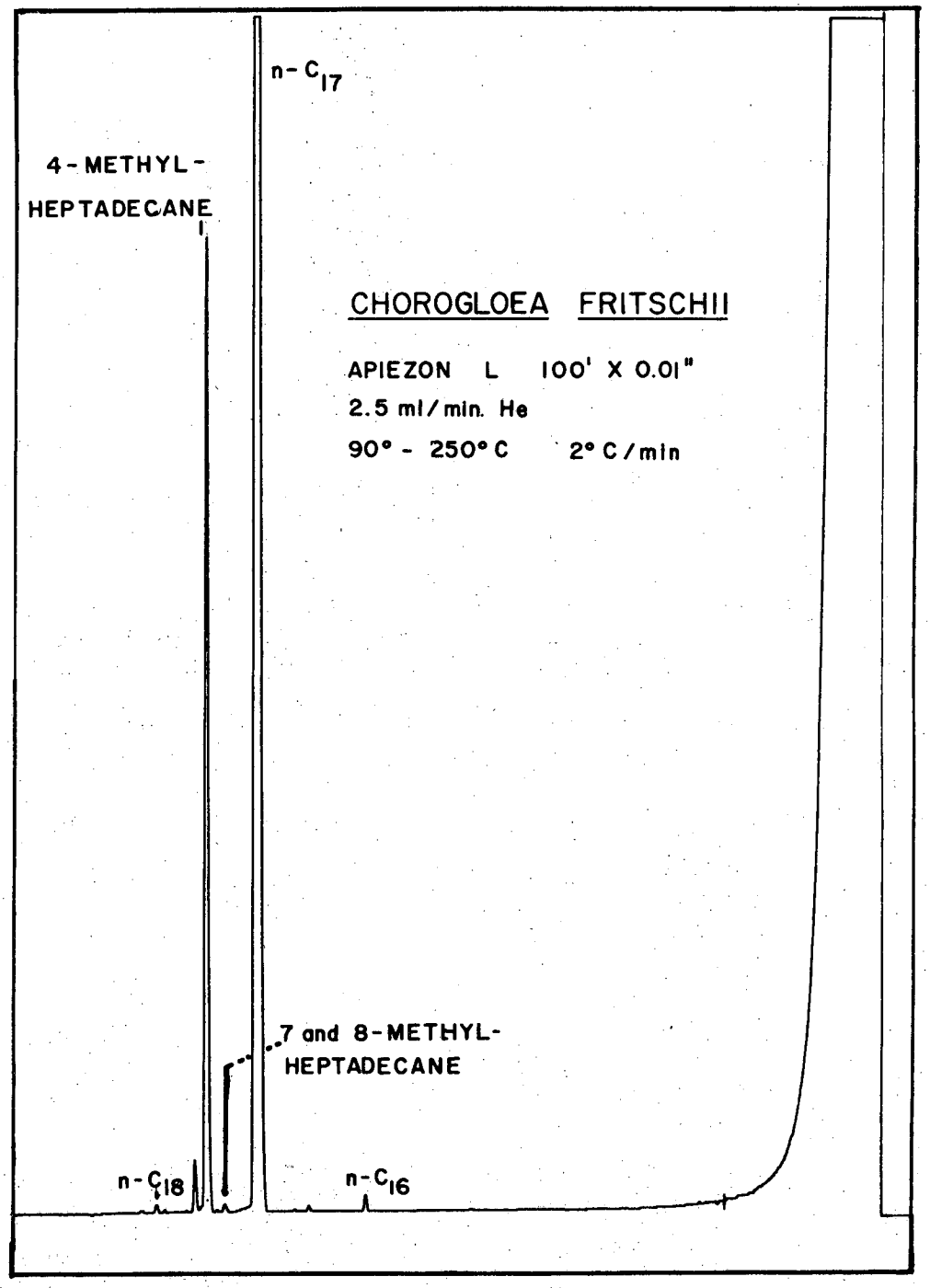
The 7-methyl and 8-methylheptadecane account for only 0.1% of the total hydrocarbons found in Chlorogloea fritschii. However, another branched hydrocarbon having a gas chromatographic retention time longer than 7 and 8-methylheptadecane but shorter than the n-C_{18} hydrocarbon accounts for 11.5% of the total hydrocarbons (Figure 19). The mass spectrum (Figure 20) suggests that it is not related to any of the common hydrocarbon structures, iso- and anteiso- forms, that have been characterized in biological sources and may have the structure 4-methylheptadeane.

Figure 18: The top figure shows the gas chromatogram of total aliphatic hydrocarbons from extractable organic material of Soudan Shale. The bottom figure shows this fraction with coinjection of branched-C_{18} hydrocarbon. All the conditions are described in the figure 6.


n-C18
n-C17
n-C19
SOUDAN
TOTAL H/C
Apiezon L
100' x 0.01"
90°-250° C
2° C / min.
2.5 ml / min. He
Pristane
Phytane
n-C16
a
n-C20
n-C15
b-C18
+ a
SOUDAN
TOTAL H/C
with coinj. of b-C18

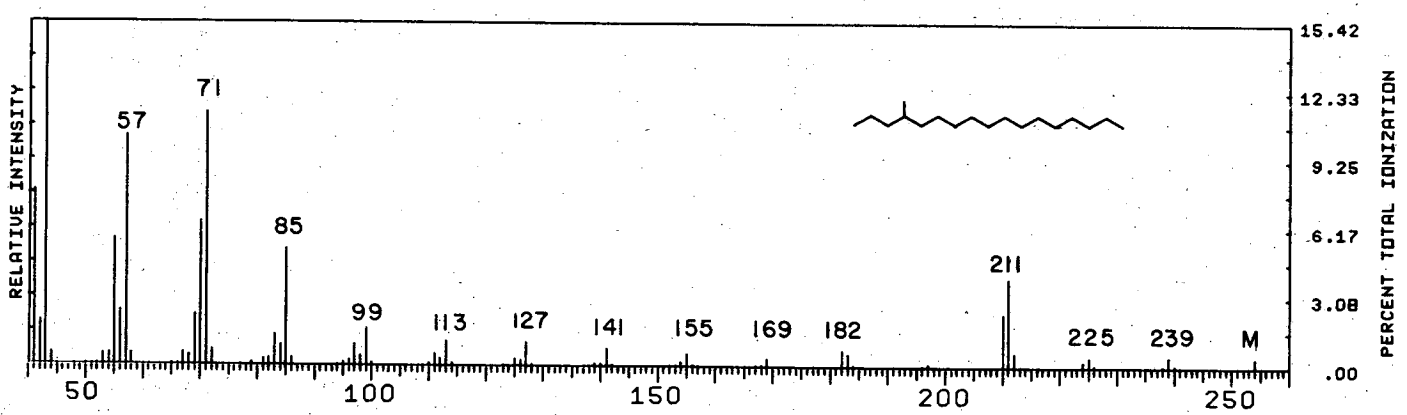
XBL 686-4231

Figure 19: Gas Chromatographic separation of Chlorogloea fritschii hydrocarbon mixture is obtained with an Aerograph-204 instrument.


n-C17
4-METHYL-
HEPTADECANE
CHOROGLOEA FRITSCHII
APIEZON L 100' X 0.01"
2.5 ml/min. He
90° - 250° C 2° C/min
7 and 8-METHYL-
HEPTADECANE
n-C18
n-C16

XBL 685-4204

Figure 20: Mass spectrum of Chlorogloea branched-C_{18} is obtained with an A.E.I. MS-12 mass spectrometer.


RELATIVE INTENSITY
PERCENT TOTAL IONIZATION
15.42
12.33
9.25
6.17
3.08
.00
57
71
85
99
113
127
141
155
169
182
211
225
239
M
50
100
150
200
250

XBL 685-4205

CHAPTER 4 REFERENCES

1. E.D. McCarthy and M. Calvin, Nature, 216, 642 (1967).
2. J. Han, E.D. McCarthy, W. Van Hoeven and M. Calvin, Proc. Nat. Acad. Sci. 59, 29 (1968).
3. J. Brand, G. Eglinton "Application of Spectroscopy to Organic Chemistry", Ch. 4, Oldbourne Press, London (1965).
4. G. Stork and S.R. Dowds, J. Am. Chem. Soc., 85, 2179 (1963).
5. G. King, J. Chem. Soc., 1826 (1938).
6. L.F. Fieser and M. Fieser, "Reagents for Organic Chemistry", J. Wiley and Sons, New York, 1967, p. 142.
7. J. Read, G.J. Robertson and A.M.R. Cook, J. Chem. Soc., 1276 (1927).
8. D. Todd, Org. Reactions 4, 378 (1948).
9. Huang-Minlon, J. Amer. Chem. Soc. 68, 2487 (1946).
10. R. Ryhage, Anal Chem. 36, 759 (1964).
11. J.T. Watson and K. Biemann, Anal. Chem. 36, 1135 (1964).
12. S. Hünig, E. Lücke and E. Benzing, Ber., 90, 2833 (1957).
13. Nguyen Dinh-Nguyen, Acta Chem. Scand. 12, 585 (1958), as modified by the insertion of additional cooling coil, as described by W. Smith "Studies in the Synthesis of Micoceranic Acid", Part II, Thesis, Oxford (1959).
14. J. Cason and H. Rapoport, "Laboratory Text in Organic Chemistry", Prentice-Hall Inc. p. 449 (1963).
15. H.C. Brown and C.A. Brown, J. Amer. Chem. Soc. 84, 1493 (1962).
16. Ibid. 84, 2827 (1962).
17. E.S. Barghoorn and S.A. Tyler, Annals N.Y. Acad. Sci. 108, 4al (1963).

18. E.S. Barghoorn and S.A. Tyler, Science 147, 3658 (1965).

19. E.S. Barghoorn, W.G. Meinschein and J.W. Schopf, Science 148, 461 (1965).

20. E.S. Barghoorn and J.W. Schopf, Science 150, 3694 (1965).

21. E.S. Barghoorn and J.W. Schopf, Science 152, 758 (1966).

22. J.W. Schopf and E.S. Barghoorn, Science 156, 508 (1967).

CHAPTER 5. BIOSYNTHESIS OF HYDROCARBONS

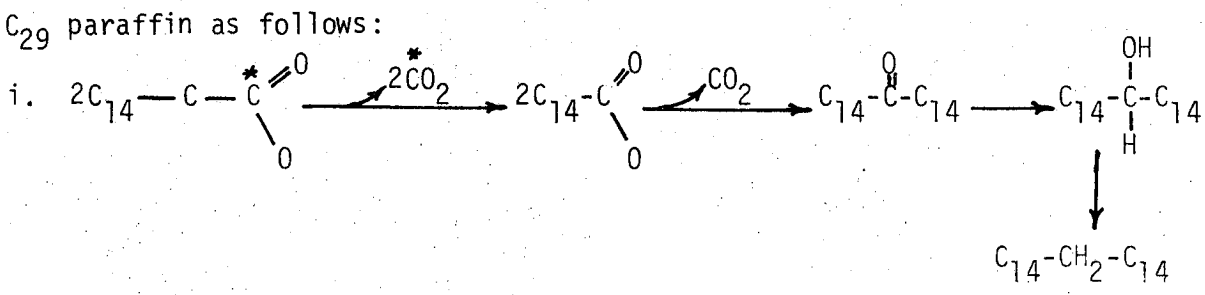
1. Introduction

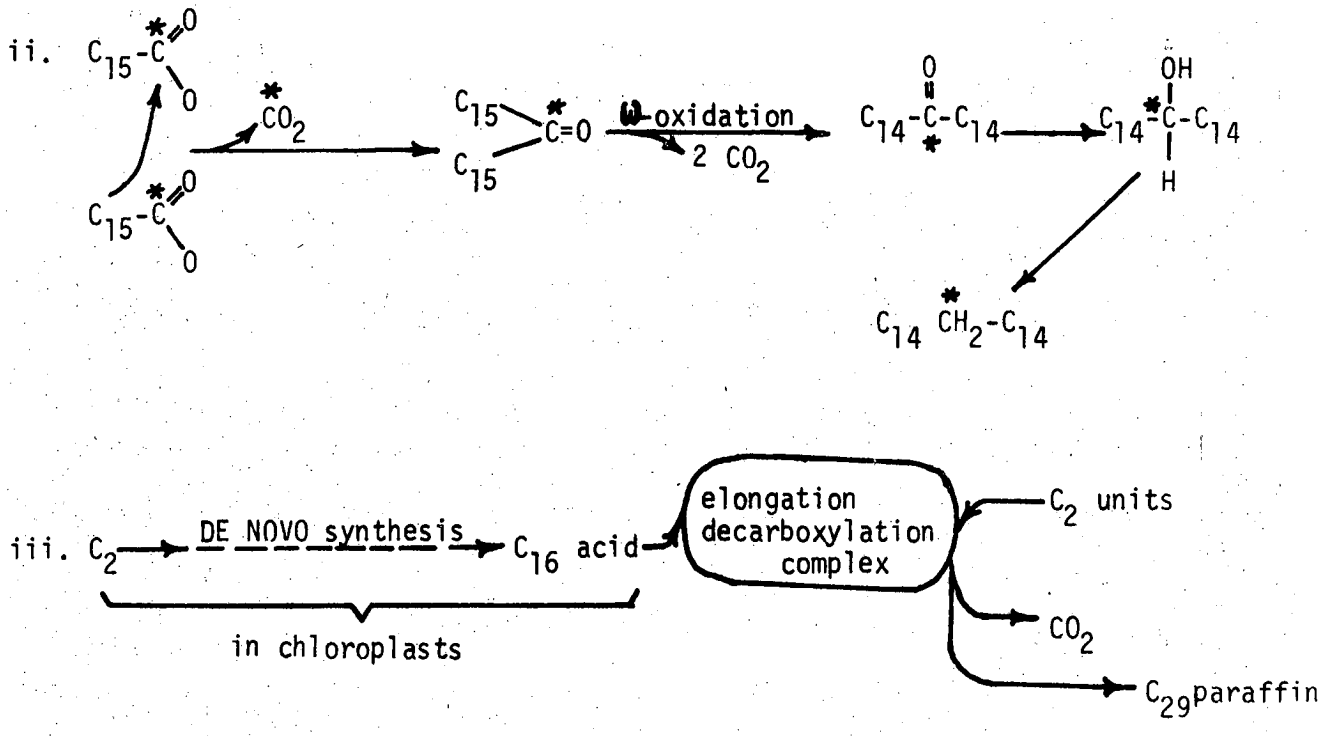
The cuticular wax of higher plants invariably contains long chain paraffins C_{21} to C_{35} in length, with C_{27}, C_{29}, and C_{31} usually predominating.[1] These paraffins can be utilized by microorganisms[2] and to a limited extent by animals[3]. Little is known about the biosynthesis of paraffins in spite of the widespread occurrence of paraffins in nature.[4,5] In most plants one paraffin is predominant; for example, in Brassica oleracea (broccoli, cabbage) more than 90 percent of the paraffin fraction is a C_{29} alkane, and in Senecio odoris it is a C_{31} alkane that predominates.[6]

The biosynthesis of hydrocarbons has been studied more than that of other waxy compounds. Nevertheless, with the exception of squalene which has been studied very extensively because of its connection with steroids[7], our understanding of the biosynthesis of hydrocarbons is still scanty.

Piek[8] reported that acetate labeled with deuterium or ^{14}C and carbon atoms of glucose were incorporated into beeswax paraffins as well as other compounds. Although paraffin synthesis also occurs in microorganisms, most of the studies were of paraffin synthesis in higher plants.

Kolattukudy[9] proposed three condensation of fatty acids pathways to form C_{29} paraffin as follows:





Since C_{16} and C_{18} acids are the most abundant acids in plants, it is reasonable to presume that n-nonacosane and n-untriacontane are formed by the condensation of these two acids respectively.

If the labeling pattern obtained in the C_{29} ketone of Brassica oleracea can be assumed to be the same as that in the C_{29} paraffin, the double-ω oxidation pathway (pathway ii) can be ruled out because if this pathway were followed the carboxyl carbon of the acetate would give rise to the carbonyl carbon of the ketone, but the methyl carbon of the acetate was shown to give rise to the carbonyl carbon.[10]

According to the condensation pathway (pathway i), the n-C_{15} acid might be expected to be incorporated into the C_{29} paraffin at least as fast as the C_{16}

acid, which should be the precursor of the C_{15} acid. However, in Brassica oleracea[9] the C_{16} acid was more readily incorporated into the C_{29} paraffin than the C_{15} acid was. Furthermore, if pathway i were followed, the carboxyl carbon of the C_{16} acid would be lost during the -oxidation process which precedes the condensation. Thus the C_{16} acid labeled with ^{14}C only in the carboxyl carbon would not be expected to give rise to radioactive C_{29} ketones or paraffins. The fact that palmitic acid-1-^{14}C was as efficient as uniformly labeled palmitic acid in providing radioactivity to the paraffin[9] showed that the carboxyl carbon of the C_{16} acid is not lost. Furthermore, palmitic acid-16-^{14}C and palmitic acid-1-^{14}C were equal in their incorporation of ^{14}C into the paraffin[11].

According to pathway iii, the product of de novo fatty acid synthesis, namely the C_{16} acid, becomes the substrate for an elongation-decarboxylation enzyme complex. This complex successively adds C_2 units until the chain length reaches the vicinity of C_{30}; then the fatty acid is decarboxylated and the C_{29} paraffin is released. Some decarboxylation must occur before C_{30} and after C_{30}, giving rise to other paraffins. The incorporation[9,10] of specifically labeled acetate into C_{29} compounds of Brassica oleracea is in agreement with the elongation pathway. As predicted by the hypothesis and as indicated by the experimental evidence, the C_{16} palmitic acid is incorporated as a unit into the paraffin.

When fatty acids increased in chain length from C_{10} to C_{14}, there was a relatively small increase in incorporation into the C_{29} compounds, but further increase in chain length of the precursor caused a rather rapid increase in

incorporation.[9,11] Stearic acid was incorporated at least twice as fast as C_{16} acid into the paraffins. The longer fatty acids (C_{16} and C_{18}) presumably can become the substrate directly for the enzyme system that synthesizes paraffins, and therefore much more readily undergo conversion into paraffins.

Distribution of aliphatic hydrocarbons in a variety of microorganisms were described in other chapters. Of all the species examined, the simplest mixture of hydrocarbons was found in the blue-green algae, e.g., Nostoc, which has only four components in the hydrocarbon fraction of extracts of the algae. The major components of the hydrocarbons are n-heptadecane (n-C_{17} hydrocarbon) and a "branched-chain C_{18} alkane" which has since been shown[12] to be an equimolar mixture of 7-methyl and 8-methylheptadecane. In addition to these hydrocarbons which constitute 99% of the hydrocarbon content of the algae, two minor components are also present, viz. n-pentadecane (n-C_{15} hydrocarbon) and n-hexadecane (n-C_{16} hydrocarbon).

The occurrence of these hydrocarbons in the blue-green algae apparently at a critical stage of evolutionary diversification led us to examine their biological origins and possible metabolism in the living cell. In this chapter we report results of experiments in the biosynthesis and metabolism of methyl-branched octadecane, n-heptadecane, and the minor constituent n-pentadecane of Nostoc.

The unique occurrence of these hydrocarbons, apparently confined to blue-green algae and appearing at a critical stage of evolutionary diversification, may be of considerable significance. It would be of interest to gain an

understanding of the biosynthetic pathway to these branched C_{18} alkanes from Nostoc in these primitive organisms. One possible precursor, 7-heptadecane or the corresponding acid, does not appear to occur in blue-green algae. An alternative source might be lactobacillic acid (11,12-methyleneoctadecanoic acid).[13,14] It is formed by the fixation of a C_1 unit derived from formate to cis-vaccenic acid.[15]

2. Experimental

Eight liters of Nostoc muscorum were grown in an inorganic medium for 4 days at 25°, under a stream of air-CO_2 (v/v 95:5) and with illumination (450 ft candles) from both sides. The culture (OD 680 ∿ 0.8) was centrifuged and resuspended in a 3-l.flask with 1.5l. of the same medium and the radioactive hydrocarbons or fatty acids were dispersed in the medium by sonication. The suspension was illuminated at 25° and under CO_2 for 2 hrs. The cells were harvested by centrifugation (10,000g) in the dark, washed by resuspension in 100 ml of distilled water, and centrifuged again. About 1.8 g of radioactive cells was collected each time after the water was removed by freeze drying. The analytical procedure was described in Chapter 2.

The ω-^{14}C fatty acids (obtained from New England Nuclear Corp.) were decarboxylated by photodecomposition of their acyl iodides followed by zinc dust reduction of the alkyl halides as described by Barton, et al.[16] In each case 0.1 mmol of the acid was used and the over-all yield as determined by the radioactivity retained in the product was 60%. The products were purified by chromatography on alumina. Uniform tritium-labeled methyl vaccenite was made in this laboratory by the Wilzbach gas exposure method. Methyl vaccenate

(10 mg) was deposited evenly on a 100-ml glass reaction tube. After evacuation to 0.1 mm, 7 Ci of tritium gas and 20 ml of oxygen gas at standard condition were introduced into the reaction tube.

Methyl tritiostearate was formed by adding tritium to the double bond, and methyl tritiovaccenate was formed by tritium substitution reactions. Liquid scintillation counting indicated that 3.1 mCi of tritium was incorporated into the total product. After hydrolysis of methyl esters 300 μCi of methyl tritiovaccenic acid was isolated by thin layer chromatography on silica gel G containing 15% of silver nitrate.[19]

3. Biosynthesis of normal hydrocarbons

Although the formation of hydrocarbons in living organisms by decarboxylation of fatty acids has been a subject of conjecture for some time,[6] little is known concerning the derivation of hydrocarbons in algae. Studies in the biogenesis of hydrocarbons in other microorganisms have shed light on their mode of formation but little positive evidence has been produced for the decarboxylation of fatty acids to yield hydrocarbons. Thus Oró, et al.,[20] examined the formation of hydrocarbons in Sarcina luteau by including a variety of ^{14}C-labeled substrates (e.g., acetate, glucose, leucine, isoleucine, bicarbonate, and both palmitic-1-^{14}C and -16-^{14}C acids) in the culture media. However, the length of incubation (48 hr) and the general nature of metabolites used led to the appearance of label in a wide variety of substances.

In an effort to study the decarboxylation of fatty acids in isolation we have used exclusively w-^{14}C-labeled acids and comparatively short incubation periods (2 hrs). The low level of incorporation into hydrocarbons obtained

under these conditions can, however, be detected and the mixture of products analyzed by the techniques of gas chromatography[4] and liquid scintillation counting.

The results of an incubation of stearic-18-^{14}C acid in a culture of Nostoc is shown in Table I. An exclusive incorporation of radioactivity into n-heptadecane is observed indicating a direct decarboxylation of stearic acid. The n-heptane fraction containing the hydrocarbons accounts for 6.2% of the total radioactivity recovered; close to the remainder of the radioactivity (92.7%) is present in the methanol fraction which contains the starting material. When palmitic-16-^{14}C acid is substituted for the radioactive stearic acid, radio-activity was incorporated into n-heptadecane as well as into n-pentadecane (Table II) in the extracted hydrocarbons. The formation of n-heptadecane is likely to be a result of chain elongation to stearic acid prior to decarboxylation. However, there appears to be appreciable incorporation (15%) into fractions of the extracts other than those containing the hydrocarbons and the fatty acids. This would indicate that the synthesis of other compounds from fragments such as acetyl-CoA would give rise to radioactivity in the benzene II fraction. Unlike the case of stearic-18-^{14}C acid there was considerable breakdown of the palmitic-16-^{14}C acid during the period of incubation.

As a means of studying the possible further metabolism of the normal hydrocarbons, synthetic n-heptadecane-^{14}C and n-pentadecane-^{14}C were also incubated with cultures of Nostoc. In contrast to the incubations of ^{14}C fatty acids, no appreciable metabolism of the radioactive hydrocarbons was

TABLE I

INCUBATION OF 18-^{14}C-STEARIC ACID

Distribution of Radioactivity in the n-heptane fraction.

Hydrogen	dpm.	% of activity in fraction
n-pentadecane	47.7	--
n-hexadecane	686	0.7
n-heptadecane	1.04×10^5	98.7
Methyl-heptadecanes	674	0.6

Distribution of radioactivity in different fractions of the extraction.

Fraction	dpm.	% of total activity extracted.
n-heptane	1.05×10^5	6.2%
Benzene I	650	0.4%
Benzene II	1.2×10^4	0.7%
Methanol	1.57×10^6	92.7%

Time of incubation = 3 hours. Amount of Stearic Acid used: 50μC, 284 mg.

Radioactivity recovered: 0.77 μC.

TABLE II

INCUBATION OF 16-^{14}C-PALMITIC ACID.

Distribution of Radioactivity in the n-Heptane Fraction			Distribution of Radioactivity in Different Fractions of the Extraction		
Hydrocarbon	dpm.	% of activity in Fraction	Fraction	dpm.	% of total activity extracted
n-pentadecane	5.32×10^4	44.2	n-heptane	3.17×10^4	67.6%
n-hexadecane	2.3×10^3	1.9	Benzene I	476	1.0%
n-heptadecane	1.8×10^4	15.0	Benzene II	1.44×10^4	30.7%
Methyl-heptadecanes	4.7×10^4	38.8	Methanol	293	0.6%

Time of Incubation: 3 hours. Amount of Palmitic Acid used: 50μC, 20mg.

Radioactivity recovered: 0.06μC.

detected after the same period of incubation (Tables III and IV). The recovered radioactivity remains essentially in the hydrocarbon fractions. Analysis of these showed the retention of radioactivity only in the peaks of the gas chromatogram corresponding to the starting materials. The small amounts of radioactivity present in other peaks of the chromatograms are due to tailing of the large quantities of the ^{14}C hydrocarbons present in each chromatogram. This was shown by injection of a similar amount of starting material and observing a similar radioactive distribution of the peaks in each case. Within experimental error therefore, the results above may be regarded as evidence for the absence of metabolism of the hydrocarbons. However, the presence of trace amounts of hydrocarbon remaining outside the algal cells even after washing with water subsequent to harvesting them cannot be excluded. The results may, therefore, only indicate the failure of the hydrocarbons to enter the algal cells.

4. Biosynthesis of 7- and 8-Methylheptadecanes

The first part of this work was a search for the presence of possible intermediates such as vaccenic acid in Nostoc. Holton, et al.,[21] reported 7.4% octadecenoic acid in the total acids of Nostoc muscorum. They reported it as Δ^{9}-C_{18} acid. However, the double bond position isomers were not separated by their packed gas-liquid chromatographic column.

Our gas chromatographic pattern for the methyl esters is given in Figure 1. The components were identified by glpc coinjections of standard esters and their mass spectra. Methyl oleate and methyl vaccenate had identical mass spectra but could be separated by a capillary column coated with Hi-Eff-4BP (polybutane-1,4-diol succinate). The fatty acid mixture contained mainly

TABLE III

INCUBATION OF n-1-^{14}C-HEPTADECANE

Distribution of Radioactivity in the n-Heptane Fraction			Distribution of Radioactivity in Different Fractions of the Extraction		
Hydrogen	dpm.	% of activity in Fraction	Fraction	dpm.	% of total activity extracted
n-pentadecane	35	--	n-heptane	4.4×10^4	93.6%
n-hexadecane	644	1.5	Benzene I	2340	5.0%
n-heptadecane	4.15×10^4	94.7	Benzene II	119	0.2%
Methyl-heptadecanes	1660	3.8	Methanol	530	1.1%

Time of incubation: 3 hours Amount of n-heptadecane used: 10μC, 16.3 mg.

Radioactivity recovered: 2.2×10^{-2} μC.

TABLE IV

INCUBATION OF n-1-^{14}C-PENTADECANE

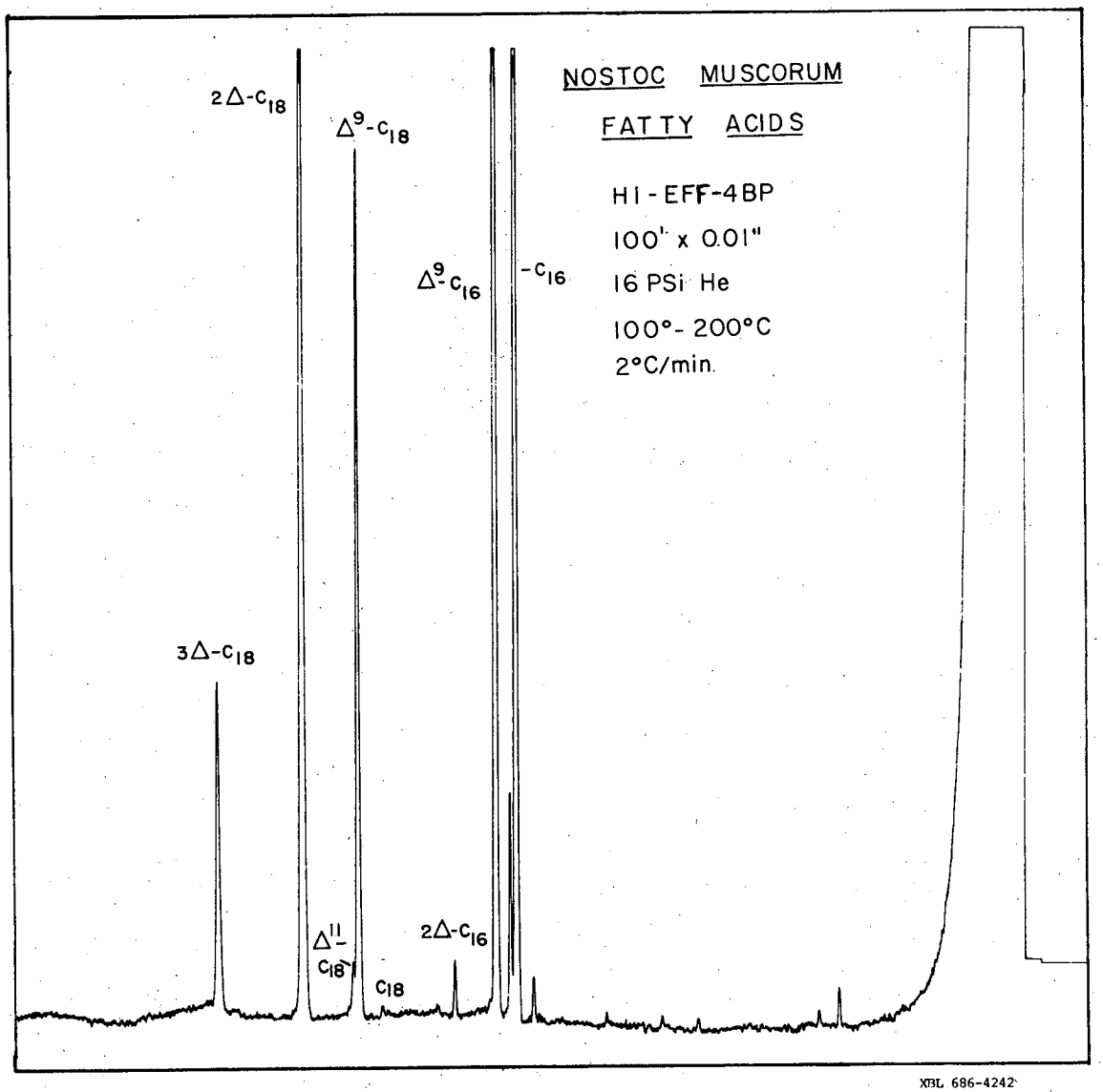
Distribution of Radioactivity in the n-Heptane Fraction			Distribution of Radioactivity in Different Fractions of the Extraction		
Hydrogen	dpm.	% of activity in Fraction	Fraction	dpm.	% of total activity extracted
n-pentadecane	5.4×10^5	95.6	n-heptane	6.35×10^5	98.7%
n-hexadecane	2.22×10^4	3.6	Benzene I	6700	1.0%
n-heptadecane	4830	0.8	Benzene II	265	--
Methyl-heptadecanes	338	--	Methanol	1340	0.2

Time of incubation: 3 hours.

Amount of n-pentadecane used: 15μC, 18 mg.

Radioactivity recovered: 0.295μC.

Figure 1: Gas chromatographatic separation of total fatty acids of Nostoc muscorum. A stainless steel column 100 ft. x 0.01 in. coated with HI-EFF-4BP. Aerograph model 204 gas chromatograph was equipped with a flame ionization detector. Helium pressure 16 psi no split. Temperature was programmed from 100°C to 200°C at a rate of 2°C/min.


NOSTOC MUSCORUM
FATTY ACIDS
HI-EFF-4BP
100' x 0.01"
16 PSi He
100°- 200°C
2°C/min.
2Δ-C18
Δ9-C18
Δ9-C16
-C16
3Δ-C18
Δ11-C18
C18
2Δ-C16

XBL 686-4242

C_{16} and C_{18} acids. The percentages of fatty acids are given in Table V.

This is the first observation of vaccenic acid (cis-Δ^{11}-C_{18}) in blue-green algae. Bloch, et al.,[22,23] considered that two different pathways exist for the synthesis of monounsaturated fatty acids. According to Bloch:[18] "among the C_{18} monosaturated fatty acids, the anaerobic bacterial pathway is apparently capable of producing both the Δ^9 and the Δ^{11} isomers, whereas the aerobic process invariably yields the Δ^9 acid." In our case, Δ^{11} is presumably produced by an aerobic process. Vaccenic acid (cis-Δ^{11}-C_{18}) could be formed as a minor product from stearic acid by a oxidative mechanism. Other possible precursors of the branched alkane such as acids with a methyl branch or cyclopropyl ring have not been found in Nostoc.

The confirmation of the presence of vaccenic acid in Nostoc led us to investigate its possible role in the biosynthesis of the methylheptadecanes. When tritium-labeled vaccenic acid was incubated with Nostoc, about 10% (1.1×10^7 dpm) of the radioactivity was incorporated into the cell and 90% remained in the supernatant solution. In the ^{14}C-labeled methionine experiment 8% (3.6×10^6 dpm) of the radioactivity was incorporated into the cell. The distribution of radioactivity in the various column chromatographic fractions of these experiments is given in Tables VI and VII.

When methionine-methyl-^{14}C was used, essentially all the radioactivity in the n-heptane fraction was incorporated in 7- and 8-methylheptadecanes. As shown in Table VI this mixture contains 9.05×10^5 dpm or 99% of the radioactivity. However, only 80.1% of the radioactivity of the n-heptane fraction was found in the branched C_{18} when tritiated vaccenic acid was used as

TABLE V

Fatty Acids Composition (%) of Total Lipids of *Nostoc muscorum*.

Saturated Acids		Mono-unsaturated Acids		Polyunsaturated Acids	
n-C_{16}	23.4	Δ^{9}-C_{16}	29.8	2Δ-C_{16}	1.5
n-C_{18}	0.6	Δ^{9}-C_{18}	13.8	2Δ-C_{18}	18.3
		Δ^{11}-C_{18}	1.2	3Δ-C_{18}	8.6

a starting material. The incorporation of radioactivity into n-heptadecane is presumably a result of hydrogenation followed by decarboxylation.

The data in Tables VI and VII also confirm the fact that the methionine methyl group is specifically added to the double bond of vaccenic acid. If a methylene group is inserted into the double bond, followed by decarboxylation and then ring opening, it could produce the 50:50 mixture of 7- and 8-methylheptadecanes.

By using methionine-methyl-^{14}C and cis-vaccenic-^{14}C acid, Liu and Hofmann[24] were able to demonstrate that lactobacillic acid (11,12-methyleneoctadecanoic acid) biosynthesis in Lactobacillus arabinosus, an anaerobic bacterium, involved the addition of a methylene group into the double bond of cis-vaccenic acid. In the methionine-methyl-^{14}C and formate-^{14}C experiments, their results showed most of the radioactivity of lactobacillic acid was on the cyclopropyl site. It is suggested that the 11,12-methyleneoctadecanoic acid could be the intermediate of the branched C_{18} hydrocarbon. We are at present investigating this possibility.

TABLE VI

INCUBATION OF METHIONINE-METHYL-C^{14}

Distribution of Radioactivity in the n-Heptane Fraction			Distribution of Radioactivity in Different Fractions of the Extraction		
Hydrogen	dpm	% of Activity in Fraction	Fraction	dpm	% of Total Activity Extracted
n-pentadecane	15	1.6×10^{-3}	n-heptane	9.52×10^{5}	26.8%
n-hexadecane	80	8.8×10^{-3}	Benzene I	7×10^{3}	0.2%
n-heptadecane	9×10^{3}	0.85	Benzene II	2.49×10^{4}	0.7%
Methyl-heptadecanes	9.05×10^{5}	99	Methanol	2.56×10^{6}	72.3%

TABLE VII

INCUBATION OF TRITIATED VACCENIC ACID

Distribution of Radioactivity in the n-heptane Fraction			Distribution of Radioactivity in Different Fractions of the Extraction		
Hydrogen	dpm	% of Activity in Fraction	Fraction	dpm	% of Total Activity Extracted
n-pentadecane	360	0.5	n-heptane	7.15×10^5	6.5%
n-hexadecane	2.2×10^4	3.1	Benzene I	2.2×10^4	0.2%
n-heptadecane	1.09×10^5	15.3	Benzene II	8.8×10^4	0.8%
Methyl-Heptadecane	5.37×10^5	80.1	Methanol	1.01×10^7	92.5%

CHAPTER 5 REFERENCES

1. G. Eglinton, A.G. Donzale, R.J. Hamilton and R.A. Raphael, Phytochem., 1, 89 (1962).

2. T.E. McKenna and R.E. Kallio, Ann. Rev. Microbiol. 19, 183 (1965).

3. P.E. Kolattudy, and L. Hankin, J. Nutr. 90, 167 (1966).

4. N. Nicolaides, J. Am. Oil Chem. Soc., 42, 691 (1965).

5. A.M. Silva Fernandez, E.A. Baker and J.T. Martin, Ann. Appl. Biol. 53, 43 (1964).

6. P.E. Kolattududy, Science, 159, 498 (1968).

7. A.A. Kuksis, Biochemistry 3, 1086 (1964).

8. T. Piek, J. Insect. Physiol. 10, 563 (1964).

9. P.E. Kolattukudy, Biochemistry 5, 2265 (1966).

10. P.E. Kolattukudy, ibid. 4, 1844 (1965).

11. P.E. Kolattududy, Plant Physiol. 43, 1466 (1968).

12. J. Han, E.D. McCarthy, M. Calvin and M.H. Benn, J. Chem. Soc. (c) 2785 (1968).

13. K. Hofmann and R.A. Lucas, J. Amer. Chem. Soc., 72, 4328 (1950)

14. K. Hofmann, G.J. Marco, and G.A. Jeffrey, J. Amer. Chem. Soc., 80, 5717 (1958).

15. K. Hofmann and T.Y. Liu, Biochim. Biophys. Acta, 37, 364 (1960).

16. D.H.R. Barton, H.P. Faro, E.P. Serebryakov, and N.F. Woolsey, J. Chem. Soc., 2438 (1965).

17. C.T. Peng, J. Phys. Chem. 70, 1397 (1966).

18. E.P. Jones, L.H. Mason, H.J. Dutton, and R.F. Nystron, J. Org. Chem., 25, 1413 (1960).

19. C.B. Barret, M.S.J. Dallas, and F.B. Padley, Chem. Ind. (London), 1050 (1962).

20. T.G. Tornabene and J. Oró, J. Bacteriol., 94, 349 (1967).

21. R.W. Holton, H.H. Blecker, and T.S. Stevens, Science, 160, 545 (1968).

22. K. Bloch, P. Baronowsky, H. Goldfine, W.J. Lennarz, R. Light, A.T. Norris, and G. Scheuerbrandt, Federation Proc., 20, 921 (1961).

23. G. Scheuerbrandt and K. Bloch, J. Biol. Chem., 237, 2064 (1962).

24. T.Y., Liu and K. Hofmann, Biochemistry, 1 181 (1962).

CHAPTER 6. CHEMICAL TAXONOMIC STUDIES OF HYDROCARBONS.

1. Introduction

Living organisms can be classified chemically in accordance with the substances made by them. Such a chemical classification may be compared with or used as a supplement to morphological classification and may be of some importance in the development of the true natural system of angiosperm phylogency.

According to Stanier[1] the evolutionary step from the procaryotic cell (blue-green algae and bacteria) to the eucaryotic cell (green algae, fungi, protozoa, higher plants and animals) is recognized by the appearance (or presence in some cases) of nuclear membrane, mitotic division, chromosome number, cytoplasmic streaming and mitochondria in the eucaryotic cell. Since lipids are important constituents of cytoplasmic and intracellular membranes, studies have been made to determine whether such evolutionary transitions are reflected at the molecular level. Very little work has been reported on evolutionary development studies of lipids. Bloch[2] reported the absence of sterols in procaryotic cells. However, Reitz and Hamilton[3] recently isolated two sterols from blue-green algae. Their results showed that very harsh methods had to be used to extract the sterols from the algae. This suggested that sterols might be bound very tightly within the procaryotic cells. Parker[4] also showed that the amount of polyunsaturated fatty acids containing two or more double bonds is very low in blue-green algae. In this chapter the chemical taxonomical approach was used to examine the hydrocarbons from different groups of microorganisms.

2. Samples and Methods

Cultures of four species of blue-green algae, seven species of non-photo synthetic bacteria, and six species of green algae and photosynthetic bacteria were extracted, fractionated and analyzed by the methods previously described. All blue-green algae, green algae, and two of the photosynthetic bacteria, Rhodospirillum rubrum and Rhodopseudomonas spheroides, were grown in this laboratory, in an inorganic medium[5] for four days at 25°C, under a stream of air-CO_2 (v/v 95:5) and with illumination (450 ft. candles) from both sides. The cultures (OD 680 0.8) were harvested and centrifuged in a continuous flow system. The wet cells were washed by resuspension in distilled water, and centrifuged again. About 0.4 g of dry cell per liter of medium was collected each time after the water was removed by freeze drying.

Two nonphotosynthetic bacteria, E. coli and Micrococcus lysodeikiticus, were purchased from Miles Laboratories Inc., Elkhart, Indiana. Three nonphotosynthetic bacteria, P. shermanii, Colstridium tetanomorphum, and Colstridium acidi-urici were supplied by Dr. H.A. Barker, Dept. of Biochemistry, University of California, Berkeley. Two samples, Desulfovibrio Essex 6 and Desulfovibrio hildenborough, were cultured in sodium lactate medium[6] by Dr. J.R. Postgate, University of Sussex, England. Rhodomicrobium vannielii was obtained from Dr. N.G. Carr, University of Liverpool, England; it was grown on a malate-glutamate medium, anaerobically in the light. Sulfur bacteria, chlorobrium was grown in this laboratory under light without air.

3. Identification of 1-Heptadecane

A mono-unsaturated C_{17} hydrocarbon was found only in algae, both blue-green and green algae, and was absent from other photosynthetic and non-

photosynthetic bacteria. The percentage of this alkene in the total hydrocarbon fractions of Anacystis nidulans (blue-green) and Chlorella pyrenoidosa (green) (Fig. 1) was 2.95 and 81.5% respectively. This alkene may be of considerable significance in green algae, since it is the predominent component in the total hydrocarbon mixture.

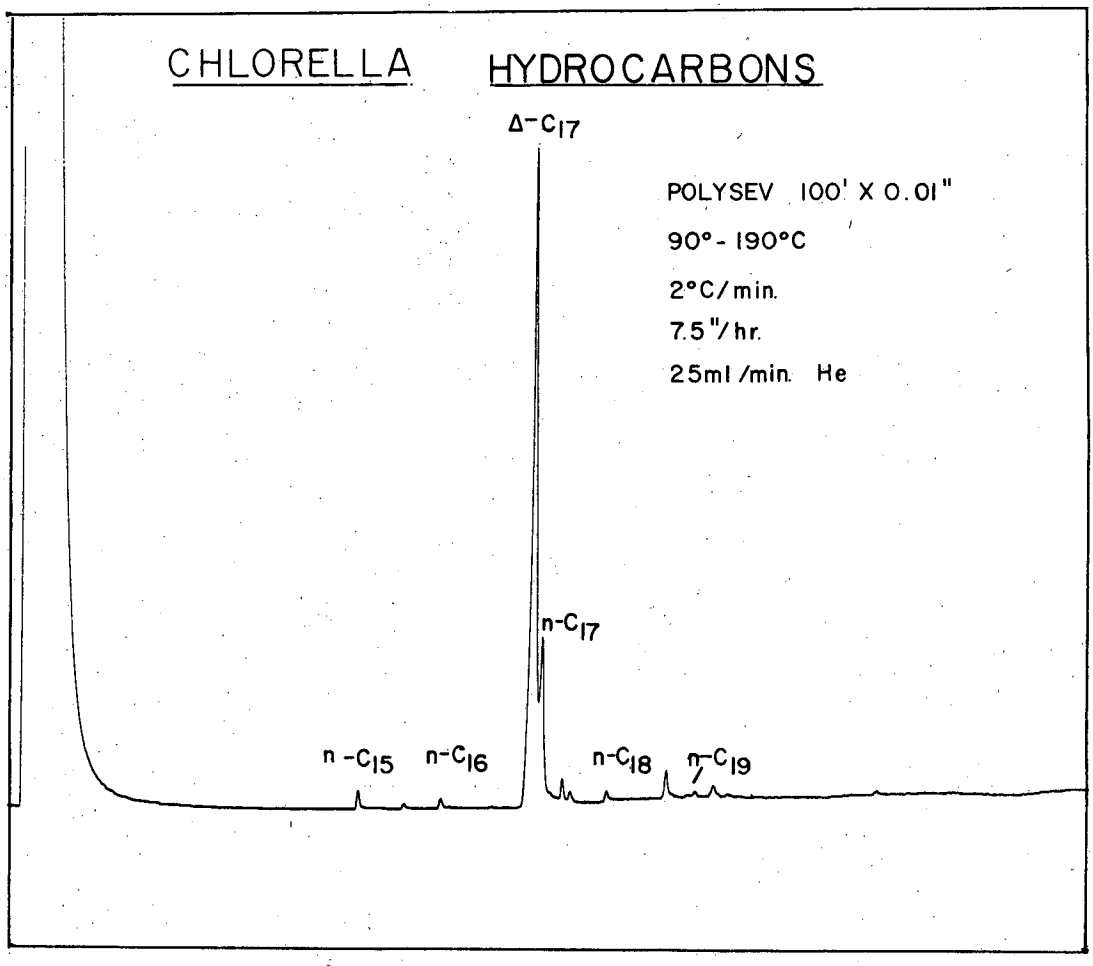
In order to determine the double bond position of Chlorella heptadecene, an oxidation reaction was carried out. After the product was purified and esterified, only one ester, methyl palmitate was determined by both gas chromatographic co-injection and mass spectrometry. Thus, only one possible structure can be drawn, the double bond is on the terminal carbon position. Mass spectra of terminal unsaturated alkenes are studied and will be discussed in Chapter 8. This series of normal alkene-1 has general characteristics, M-28 fragments are outstanding. The experimental procedures were as follows:

Heptadecene (5 mg) was isolated from the normal heptane fraction of Chlorella pyrenoidosa by using preparative gas chromatography. The alkene was treated with an excess of chromic acid in acetic acid for two hours. After the solvent was evaporated, the product was purified with column chromatography. The methyl ester was obtained according to the procedure described in Chapter 2. Both mass spectrometry and gas chromatography indicated the final product was a single compound, C_{16} methyl ester.

4. Results and discussion

Normal paraffins are among the most stable of all biogenic organic compounds and are thought to be diagnostic of biologically produced organic matter which can be derived from the decarboxylation of fatty acids. The

Figure 1: Gas chromatographic separation of total aliphatic hydrocarbons from chlorella. Stainless steel tubing 100 ft x 0.01 in. inside diameter, coated with polysev. Aerograph model 204 gas chromatograph equipped with a flame ionization detector. Helium pressure 20 psi, no split. Temperature programmed from $90^{o}C$-$190^{o}C$ at $2^{o}C$/min.


CHLORELLA HYDROCARBONS
Δ-C17
POLYSEV 100' X 0.01"
90°- 190°C
2°C/min.
7.5"/hr.
25ml/min. He
n-C17
n-C15
n-C16
n-C18
n-C19

XBL 670-6210

normal heptadecane is the dominant compound in the hydrocarbon fraction of all the photosynthetic microorganisms, blue-green algae, green algae, and photosynthetic microorganisms, blue-green algae, green algae, and photosynthetic bacteria. But the predominance is not found in aerobic and anaerobic nonphotosynthetic bacteria. Johns et al.[7] reported that the n-C_{17} alkane was a major peak in the total normal alkane fraction of the Soudan Shale (2.5 x 10^9 years). This may indicate that photosynthetic microorganisms are a major hydrocarbon source in the Soudan Shale.

Normal alkanes of carbon number less than C_{14} and more than C_{23} are rarely present to any appreciable extent in blue-green algae and green algae(Table I). However, the hydrocarbons from photosynthetic and non-photosynthetic bacteria range in chain length from C_{13} to C_{31} (Tables II and III). These hydrocarbon distributions are very different from the higher plants which contain normal hydrocarbons ranging from C_{23} to C_{35}.[8] The difference between the normal hydrocarbon patterns of microorganisms and higher plants is striking, and there appears to be a future for taxonomic correlation based on this approach.

In the recent lake sediment, the Mud Lake of Florida (5000 years),[9] n-C_{29} and n-C_{31} alkanes are the most important peaks in the total hydrocarbon fraction. The Green River Formation (50 x 10^6 years) has two peak maxima in the normal alkane fraction[7], one at n-C_{17} and another one at n-C_{29} and n-C_{31}. It appears that the high molecular weight odd numbered paraffins, n-C_{27}, C_{29} and n-C_{31} are contributed by higher plants. In the Green River Formation the higher plants and photosynthetic microorganisms appear to play equally important roles in hydrocarbon production.

TABLE I

Hydrocarbons from Algae

	Nostoc muscorum (25) (blue-green)	*Anacystis nidulans* (25) (blue-green)	*Phormidium luridum* (25) (blue-green)	*Chorogloea fritschii* (26) (blue-green)	*Chlorella pyrenoidosa* (25) (green algae)
n-C_{15}	0.35	20.60	--	--	0.13
n-C_{16}	0.35	2.50	--	0.26	0.073
Δ^1-C_{17}	--	2.95	--	--	81.5
n-C_{17}	82.75	73.75	96.00	87.30	18.0
7- and 8-methyl-heptadecane	16.10	0.15	4.00	0.09	--
4-methyl-heptadecane	--	--	--	12.20	--
n-C_{18}	0.41	--	--	0.09	0.055

TABLE II

Hydrocarbons from Nonphotosynthetic Bacteria

	E. coli (aerobic) (27)	P. shermanii (anaerobic) (29)	Micrococcus lysodeikiticus (27)	Clostridium tetanomorphum (29)	Clostridium acidi-urici (29)	Desulfovibrio Essex 6 (30)	Desulfovibrio Hildenborough (30)
n-C_{15}	0.5	2.1	8.7	1.6	1.0	0.3	0.3
n-C_{16}	1.7	2.6	8.0	2.1	14.4	0.3	0.3
Pristane	--	46.5	2.5	3.5	2.1	0.5	0.7
n-C_{17}	5.5	13.3	8.1	9.1	50.0	1.5	2.3
Phytane	--	1.0	1.9	2.5	1.3	0.3	0.3
n-C_{18}	27.6	3.6	5.0	7.2	4.5	2.7	3.3
n-C_{19}	12.0	3.8	12.5	7.4	4.9	16.3	10.4
n-C_{20}	10.0	3.8	5.0	5.5	3.1	34.0	16.5
n-C_{21}	5.5	4.2]	2.9	4.7	1.9	26.4	11.3
n-C_{22}	6.0	4.1	0.9	5.1	1.7	9.2	5.0
n-C_{23}	8.3	3.1	0.5	6.7	1.0	2.9	3.2
n-C_{24}	7.4	1.5	0.4	7.1	1.0	1.3	4.6
n-C_{25}	6.0	1.0	0.1	5.8	0.7	0.7	7.9
n-C_{26}	3.3	0.5	--	5.0	0.5	0.7	13.3
n-C_{27}	3.3	0.5	--	4.3	--	0.5	10.8
n-C_{28}	0.5	--	--	0.9	--	0.5	5.0

TABLE II (cont'd)

Hydrocarbons from Nonphotosynthetic Bacteria

	E. coli (aerobic) (27)	*P. shermanii* (anaerobic) (29)	*Micrococcus lysodeikiticus* (27)	*Clostridium tetanomorphum* (29)	*Clostridium acidi-urici* (29)	*Desulfovibrio* Essex 6 (30)	*Desulfovibrio* Hildenborough (30)
n-C_{29}	1.4	--	--	--	--	--	--
n-C_{30}	--	--	--	--	--	--	1.1
n-C_{31}	--	--	--	--	--	--	0.5

TABLE III

Photosynthetic Bacteria

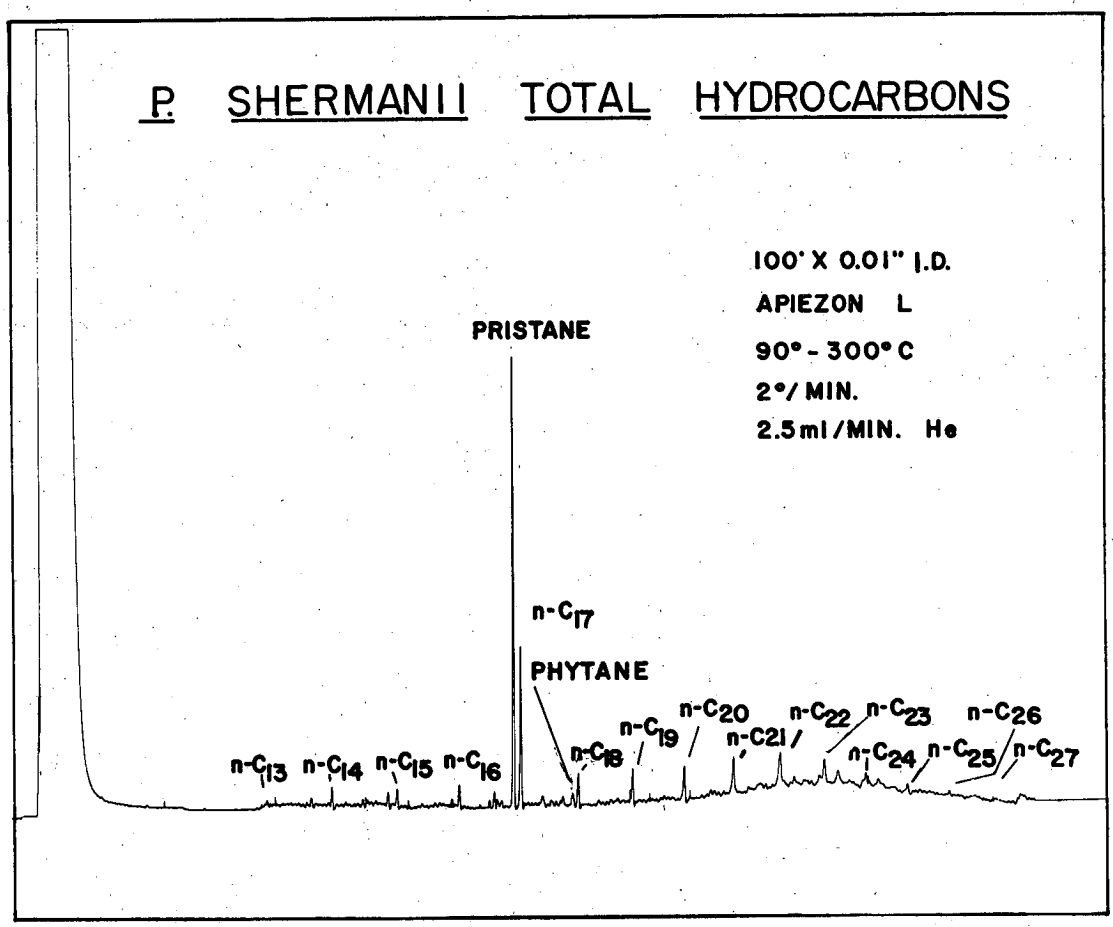
	Rhodospirillum rubrum (25)	Rhodopseudomonas spheroides (25)	Chlorobrium (sulfurbacteria) (31)	Rhodomicrobium vanniellii (28)
n-C_{15}	0.01	0.87	1.5	0.03
n-C_{16}	0.06	3.1	0.75	0.05
Pristane	0.10	9.6	0.5	0.14
Δ^1-C_{17}	--	--	--	--
n-C_{17}	3.50	42.5	50.0	0.15
Phytane	--	1.3	0.5	0.16
n-C_{18}	0.35	19.2	1.3	0.37
n-C_{19}	0.45	18.8	1.3	0.45
n-C_{20}	0.32	0.38	1.0	0.46
n-C_{21}	0.24	--	1.5	0.46
n-C_{22}	--	--	3.0	0.62
n-C_{23}	--	--	4.1	1.0
n-C_{24}	--	--	6.9	1.2
n-C_{25}	--	--	10.8	0.92
n-C_{26}	--	--	13.1	0.50
n-C_{27}	--	--	2.1	0.38
n-C_{28}	--	--	0.5	0.15
Squalene and high M.W. cyclic hydrocarbons	94.7	--	--	92.5

If we disregard the n-C_{17} hydrocarbons, the amounts of odd-numbered homologues and the even-numbered ones in either algae or bacteria are about equal (Tables I, II, and III), in contrast to higher plants[8,10]. Since the alkanes can be assumed to be end products of the living organism metabolism,[11] this may be significant in terms of the specificity of the enzyme systems which are involved in the formation of the alkanes from acetate units. It is probable that one route to the living organism hydrocarbons involves decarboxylation of the corresponding long-chain fatty acids. The decarboxylation of the fatty acids of chain length C_{10}-C_{34} which might give rise to the typical n-alkanes are dependent on the enzyme specificity of the organisms.

The 50:50 mixture of 7- and 8-methylheptadecane[12-14] appeared to occur uniquely in the blue-green algae and was absent in other photosynthetic and nonphotosynthetic microorganisms. These methyl branched alkanes seemed to be of particular significance because the methyl branches are not the iso- and anteisostructures which have been found in many living organisms. This pair of hydrocarbons may be of considerable importance in taxonomic studies, not only because of the large amount found in the blue-green algae, but also because of its unique presence in such a primitive microorganism.

The isoprenoid hydrocarbons are absent in blue-green algae and green algae, but they are widely distributed in most of the photosynthetic and nonphotosynthetic bacteria. The highest content of pristane was found in P. shermanii, (46.5%) (Fig. 2), an anaerobic nonphotosynthetic bacteria. This implies the anaerobic nonphotosynthetic bacteria has the enzymatic system which produces the isoprenoid hydrocarbons although the chlorophylls

Figure 2: Gas chromatographic separation of total aliphatic hydrocarbons from *P. shermanii*. Stainless steel tubing 100 ft. x 0.01" in inside diameter, coated with Apiezon L. Perkin-Elmer model 900 gas chromatograph equipped with a flame ionization detector. Helium pressure, 20 psi, no split. Temperature programmed from 90° to $300^{o}C$ at $2^{o}C/min$.



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are absent. This indicates the nonphotosynthetic anaerobic bacteria activity may play an important role in isoprenoid hydrogen diagenesis.

The existence of anaerobes has for years alerted scientists to the non-essential character of oxygen-based respiration, but anaerobic primary-producing systems are few. The sulfuretum, the anaerobic microbial eco-system based on the sulfur bacberia, is one such system and paleochemical evidence, based on fractionation of the sulfur isotopes, has shown that sulfureta were active at least as far back as 2×10^9 years ago. Basically, such a system requires only the intervention of sulfate-reducing bacteria and Thiorhodaceae; very reasonable arguments have been presented by Peck[15] and by Klein and Cronquist[16] that representatives of these were the dominant life forms during the anaerobic phase of life on this planet. A number of biochemical characteristics they possess may justly be held to be primitive: hydrogenase, ferroxdoxin, a phosphoroclastic system of pyruvate metabolism, CO_2 fixation by a reductive carboxylic acid cycle, a capacity for nitrogen fixation, use of APS as an "energy-rich" intermediate. It is reasonable to propose that Thiorhodaceae are models of the evolutionary precursors of green plant photosynthesis and relics of sulfur-based photosynthesis seem still to be detectable in green plants.[17] Desulfovibrio similarly seems to be a model for the antecedants of cytochrome-base aerobic life, being the only obligate anaerobe with a cytochrome concerned in respiratory chain phosphorylation. Klein and Cronquist even place Desulfovibrio as an evolutionary precursor of photosynthetic bacteria. Thus, Desulfovibrio could be the precursor of Chlorobium, a photosynthetic sulfur

bacteria. Compare the total hydrocarbon constituents of Desulfovibrio (Fig. 3) and Chlorobium, (Fig. 4) both of them have a hydrocarbon range from C_{15} to C_{28} and a single maxima at C_{20} and C_{26} respectively. However, all the photosynthetic microorganisms which include Chlorobium have a characteristic in common, the predominance of the n-C_{17} hydrocarbon.

It has been suggested[18-21] that in the early period of Precambrian time no living organisms existed on the earth except procaryotic cells, bacteria and blue-green algae. Probably after the blue-green algae began to decline, bacteria became more active. The isoprenoid hydrocarbons, higher molecular weight hydrocarbons, and those unresolved branched and cyclic hydrocarbons were produced by anaerobic nonphotosynthetic bacteria.

Figure 5, the total hydrocarbons from Clostridium tetanomorphum H-1, shows the distribution of normal alkanes and more than a hundred resolved peaks between n-C_{16} and n-C_{26}. Mass spectra indicate these unresolved peaks are branched and cyclic hydrocarbons, which also existed in every Precambrian rock.

As shown in Table III, some of the photosynthetic bacteria, Rhodospirillum rubrum and Rodomicrobium vanniellii, contain approximately 92-95% branched and cyclic hydrocarbons in the total hydrocarbon mixture. Those have a gas chromatographic retention time n-C_{27} and n-C_{32}. One predominant peak exists on each gas chromatogram. The mass spectroscopic data indicates the major peak in both cases is squalene. Approximately 1% of the steranes and triterpanes can also be detected by GC-MS. The individual structures of these cyclic hydrocarbons are still under investigation. These high molecular weight branched and cyclic

Figure 3: Gas chromatographic separation of total aliphatic hydrocarbons from Chlorobium. All the conditions are the same as Fig. 2.

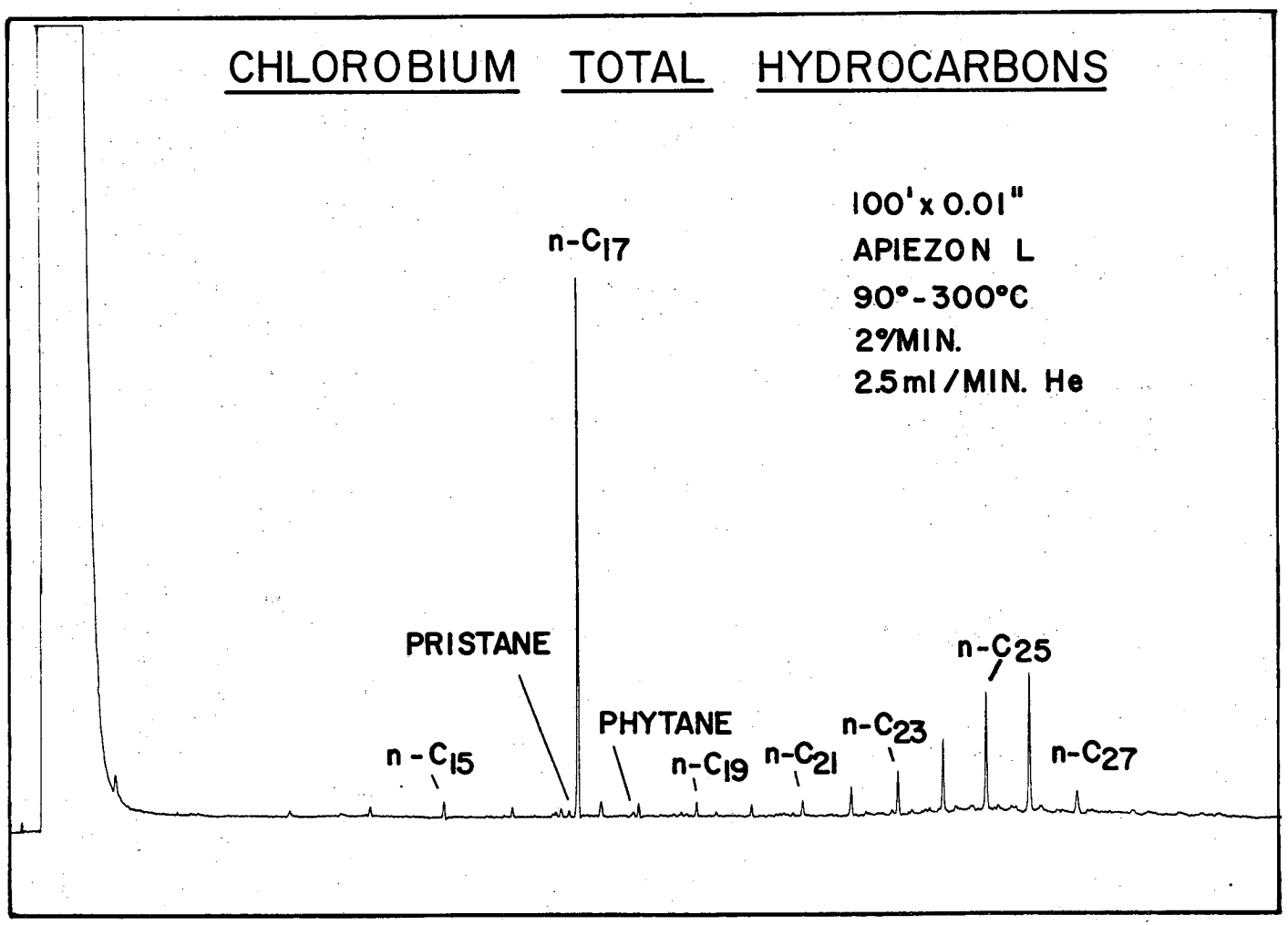

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Figure 4: Gas chromatographic separation of total aliphatic hydrocarbons from Desulfovibrio Desulfuricans Essex 6. All the conditions are the same as fig. 2.

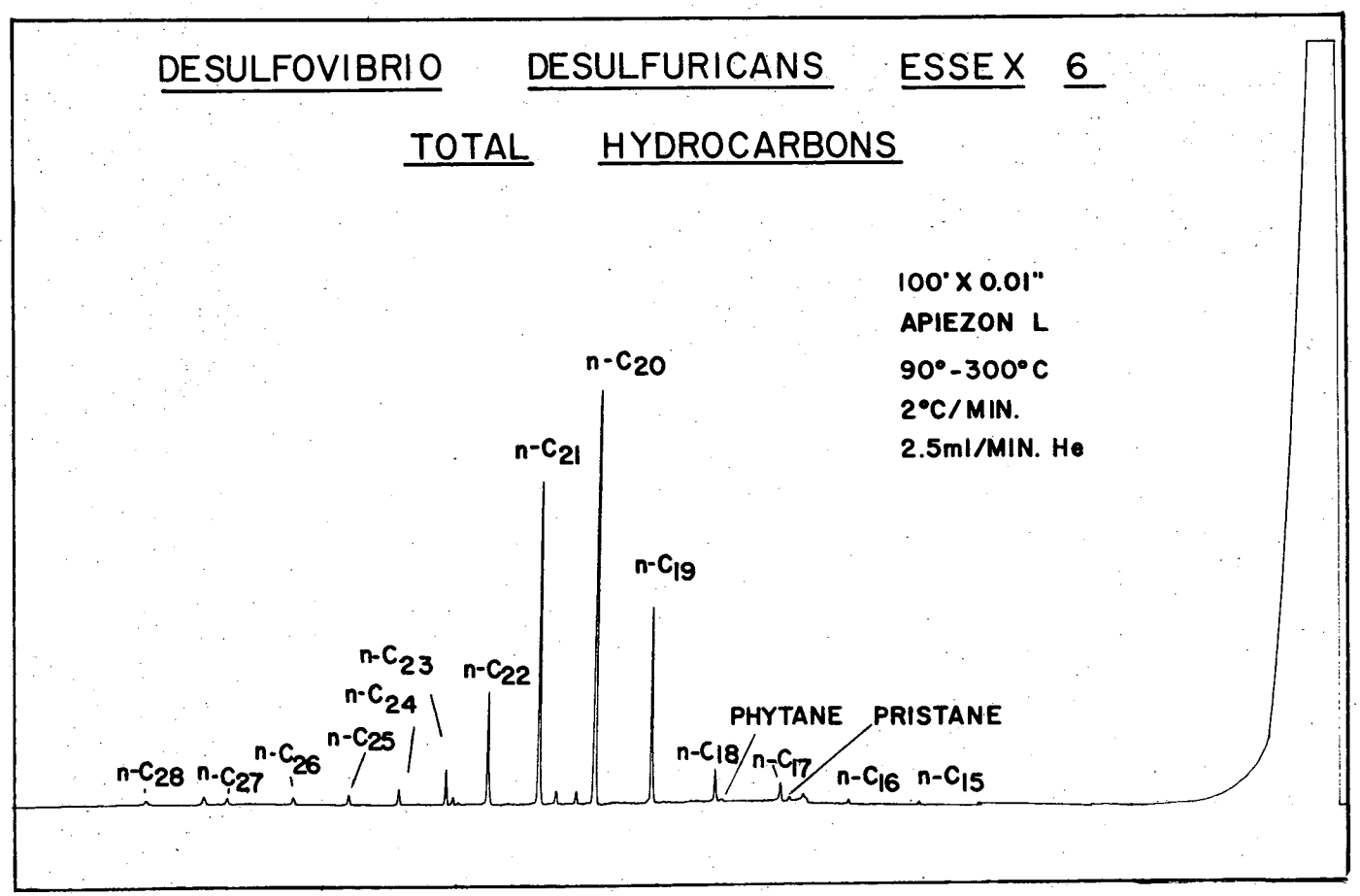

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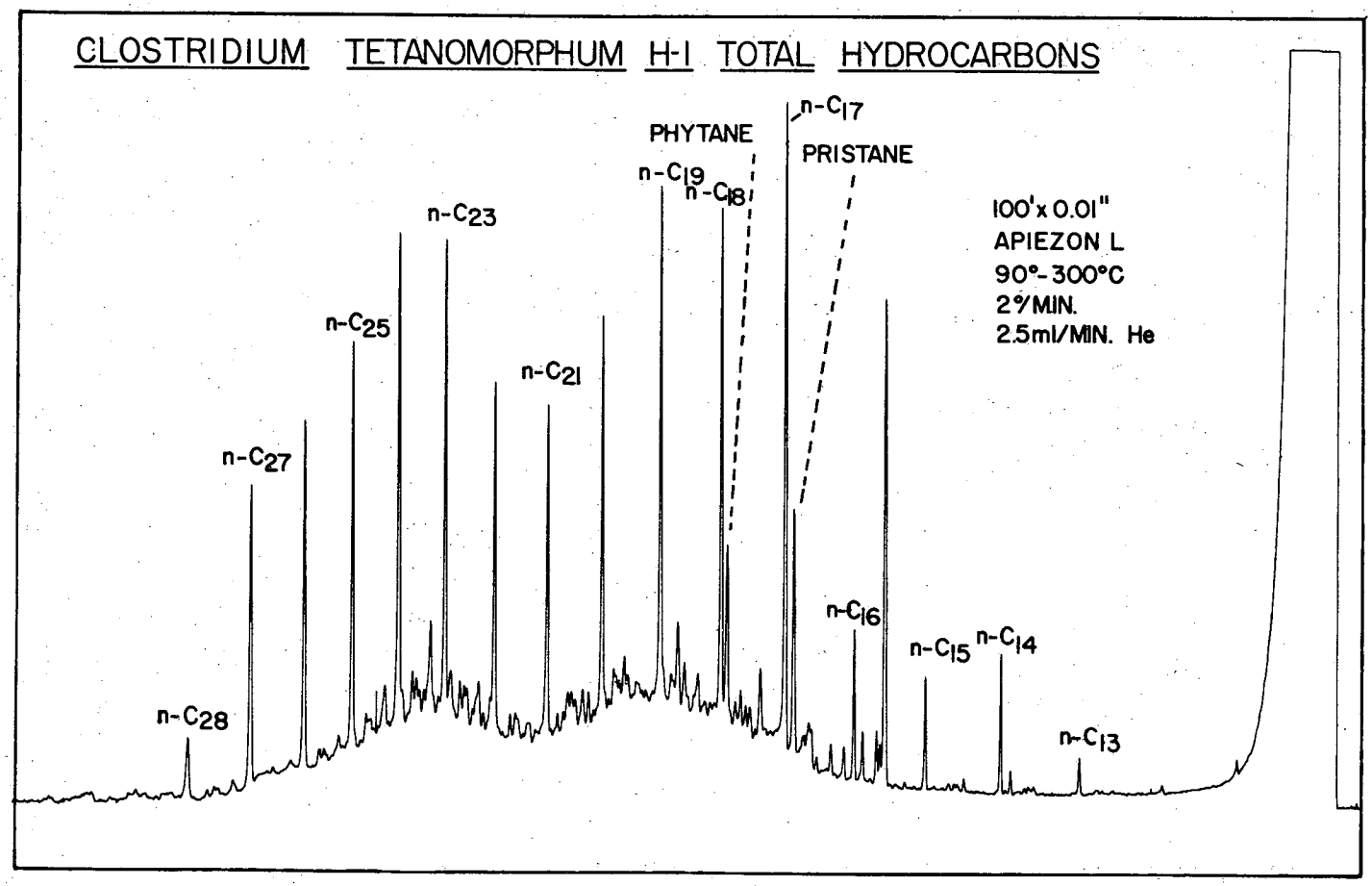
Figure 5. Gas chromatographic separation of total aliphic hydrocarbons of clostridium Tetanomorphum H-1. All the conditions are the same as Fig. 2.



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compounds are not present in any significant amount in any other procaryotic cells.

The abundance of the steranes and triterpanes is a striking feature of the total hydrocarbon content of the Green River Shale,[7] but they are only present in the Nonesuch and Soudan Shales in very small amounts.[22,23] It is probable that one route to the high molecular weight cyclic hydrocarbons involves some reactions of the sterols. The sterols which may give rise to the typical steranes and triterpanes by geological processes are common constitutents of eucaryotic cells. It is suggested that the initial reaction process has to take place by reaction of the functional groups of sterols. This could then be followed by reduction, isomerization, thermal cracking, as well as anaerobic bacterial activities during geological time to give high molecular weight cyclic hydrocarbons in the Green River Shale.

The use of chemical constitutents and so-called molecular characteristics as an aid to the classification of living organisms is not only a familiar concept now, but also a very useful tool. These characteristics have the advantage over morphological ones that they can be very exactly described in terms of definite chemical structures. The elucidation of the structures and configurations of secondary organic products can lead to an understanding not only of their biosyntheses, but of possible subsequent diagenetic changes as well. Erdtman[24] has pointed out that the most valuable substances taxonomically are not those which are involved in primary metabolic processes but rather those which are relatively stable secondary products. The hydrocarbons meet this requirement and also represent the most easily analyzed chemical substances in living organisms. The distribution of hydrocarbons ranging from C_{10} to C_{35} has been discussed previously in this report and the generalized results are shown in Table IV.

TABLE IV

The Chemical Taxonomic Relationship of Hydrocarbons Present in Living Organisms

	$n\text{-}C_{25}$, $n\text{-}C_{27}$, and $n\text{-}C_{29}$	$n\text{-}C_{17}$	Branched C_{18}	Normal H/C Distribution	Pristane and Phytane	Squalene and High M.W. cyclic H/Cs	Odd/Even H/Cs
Anaerobic Nonphotosynthetic Bacteria	within normal distribution range		absent	$C_{12}\text{-}C_{31}$+	present	absent	1
Aerobic Nonphotosynthetic Bacteria	"		absent	$C_{14}\text{-}C_{29}$	absent	absent	1
Photosynthetic Bacteria	"		absent	$C_{14}\text{-}C_{29}$	present	present in some cases	1
Blue-Green Algae	absent	>80%	present	$C_{15}\text{-}C_{20}$	absent	absent	1-5*
Green Algae	absent	>80%	absent	$C_{15}\text{-}C_{20}$	absent	absent	1-5*
Higher Plants	dominant	generally absent	absent	$C_{23}\text{-}C_{35}$	generally present	generally	>10

* The $n\text{-}C_{17}$ hydrocarbon is excluded.

\+ In some cases, many unresolved branched and cyclic hydrocarbons stay together in GC, one or two humps are shown under those sharp normal hydrocarbon peaks.

5. Hydrocarbon Constituents of Synchronized Algae

Synchronized algae, *Chlorella pyrenoidosa*, has been grown in this laboratory. A 26 hour light phase was followed by a 12 hour dark period. During the light period, the photosynthesis is taking place and the cell's volume is increasing eightfold in size. Then the cell stops growing but it divides into eight small new cells at the end of the dark period. It has been reported[9] that more than 95% of the total hydrocarbon fraction of *Chlorella pyrenoidosa* is *n*-heptadecene and *n*-heptadecane. The ratio of unsaturated to saturated compounds is 4:5.

The hydrocarbons have been analyzed in each of the growing stages in order to understand more about physiological biosynthesis of algae. The ratio of *n*-heptadecene to *n*-heptadecane is 3:5 in the dark phase, (Table V) but when the light period begins it suddenly jumps to 7:5 and stays constant through this period. Another minor peak, *n*-heptadecadene appears in the first 16 hours of the light phase, but it was not found in significant amounts either in the stage one hour before the dark phase or during the dark period.

It is suggested that strongly oxidizing conditions exist during the light phase while the cell's body is growing. Unsaturated hydrocarbons could be produced either by an oxidation-dehydration pathway or by a dehydrogenation procedure in that period. Other types of compounds found in synchronized algae, such as fatty acids and amino acids, are under investigation.

6. Summary

The chemical taxonomic features are summarized as follows:

(a) The mixture of 7- and 8-methyl heptadecane is only present in blue-green algae and absent in any other living organism so far examined.

TABLE V

Synchronized Algae, *Chlorella pyrenoidosa*

Time of Growing (hours)	Δ-C_{17}/n-C_{17} ratio
0	2.3
8	8.0
16	7.0
25	7.3
30	3.5

(b) The isoprenoid hydrocarbons, pristane and phytane are absent in algae and aerobic bacteria, but are generally present in the others.

(c) Squalene, steranes, and triterpanes are absent in algae and nonphotosynthetic bacteria but are present in most cases in photosynthetic bacteria and higher plants.

(d) In all cases, the odd over even carbon-numbered n-paraffins is approximately 1.0 in aerobic and anaerobic bacteria, while it is greater than 10 in higher plants. The value is 1.0 to 5.0 in algae when the predominant component n-C_{17} is excluded.

(e) The n-C_{17} hydrocarbon is predominant in algae, the dominance is decreased in photosynthetic bacteria. The intensity is not outstanding in nonphotosynthetic bacteria and generally absent in higher plants.

(f) The n-C_{27}, n-C_{29} and n-C_{31} alkanes are the major constituents in higher plants, within the normal distribution range in bacteria, but they are absent in algae.

CHAPTER 6 REFERENCES

1. R.Y. Stanier and C.B. Van Niel, Archiv fur Mikrobiologie 42, 17 (1962).
2. K. Bloch, in "Evolving Genes and Proteins," Eds. V. Bryson and H.J. Vogel, Academic Press, New York (1965).
3. R.C. Reitz and J.G. Hamilton, Comp. Biochem. Physiol. 25, 401 (1968).
4. P.L. Parker, C. Van Baalen, L. Maurer, Science 155, 707 (1967).
5. M.B. Allen and D.I. Arhon, Plant Physiol., 39, 3066 (1953).
6. R.J. Postgate "Laboratory Practice," 15, 1239-1244 (1966).
7. R.B. Johns, T. Belsky, E.D. McCarthy, A.L. Burlingame, P. Haug, H.K. Schnoes, W. Richter and M. Calvin, Geochem. et Cosmochim Acta 30, 1191 (1966).
8. G. Eglington, and R.J. Hamilton, Chemical Plant Taxonomy (London and New York, Academic Press, Chapter 8 (1963).
9. J. Han, E.D. McCarthy, W. Van Hoeven, M. Calvin, W.H. Bradley, Proc. Natl. Acad. Sci. 59, 29 (1968).
10. K. Stransky, M. Streibl and V. Herout, Coll. Czech. Chem. Comm. 32, 3213 (1967).
11. J. Han, H. Chan and M. Calvin, J. Amer. Chem. Soc., in press (1969).
12. J. Han, E.D. McCarthy, M. Calvin and M.H. Benn, J. Chem. Soc. (C), 2785 (1968).
13. K. Winters, P.L. Parker, and C. Van Baalen, Science 163, 467 (1969).
14. E.D. McCarthy, J. Han, and M. Calvin, Anal. Chem. 40, 1475 (1968).
15. H.D. Peck, Bact. Rev. 26, 67 (1962).
16. R.M. Klein and A. Cronquist, Quart. Rev. Biol. 42, 105 (1967).
17. K. Knobloch, Planta 70, 73 (1966).
18. J.W. Schopf and E.S. Barghoorn, Science 156, 508 (1967).

19. E.S. Barghoorn and J.W. Schopf, Science 152, 758 (1966).

20. E.S. Barghoorn, W.G. Meinschein, and J.W. Schopf, Science 148, 461 (1965).

21. E.S. Barghoorn and S.A. Tyler, Science 147, 3658 (1965).

22. W.G. Meinschein, Bull. Am. Asso. Petrol. Geologists, 43, 925 (1959).

23. P.A. Haug, Applications of Mass Spectrometry to Organic Geochemistry, Ph. D. Thesis, Univ. of California, Berkeley (1967).

24. H. Erdtman, "Perspectives in Organic Chemistry", Ed. A. Todd, Interscience, New York, p. 473 (1956).

CHAPTER 7 CHEMICAL TAXONOMIC STUDIES OF FATTY ACIDS

1. Introduction

Although lipids occur in all species of living organisms examined, a survey of the fatty acids composition has barely been started, and it is only seed fats which received a reasonably broad coverage. Even at the present time, data from other parts of the plant kingdom are fragmentary. As in the animal world, the lipids of aquatic species appear to have a different fatty acid make up from those of terrestrial species. It is convenient to start with an examination of the type and distribution of fatty acids in microorganism and then to proceed to those higher plants and animals.

2. Fatty acid constituents and biosynthesis in microorganisms

The distribution and biosynthesis of fatty acids in microorganisms have been studied for years. It has been established that straight chain saturated fatty acids are synthesized from acetyl CoA.[1,2] The acid is elongated by adding C_2 units until the chain length reaches C_{16} or C_{18}.

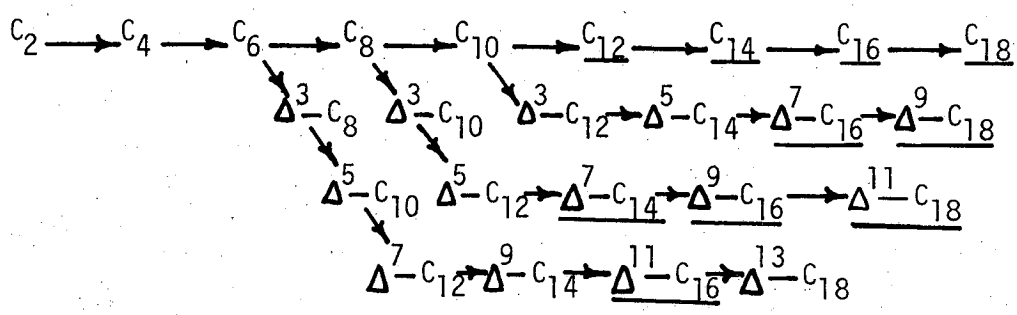
Many laboratories[3-9] have shown that linoleic and linolenic acids make a significant contribution in the total acid mixtures in photosynthetic plants and algae. But, they are not universal components of the microorganisms. *Anacystis nidulans*, a green algae,[10] does not have linolenic acid. The C_{16} polyunsaturated acid and linoleic acid are low as well. These data are similar to the photosynthetic bacteria which also do not have polyunsaturated fatty acids.[11,12] One degree of unsaturated fatty acids are the major components in photosynthetic bacteria.

Bloch[13-15] suggested the biosynthetic route to unsaturated fatty acids in microorganisms, which could be summarized as follows:

a. There are two separate pathways to synthesize monounsaturated fatty

acids. One of these produces oleic (Δ^9-C_{18}) and palmitoleic (Δ^9-C_{16}) acids by oxidative desaturation of stearic and palmitic acids respectively. It is the only or predominant pathway in yeast, blue-green algae, and in some aerobic bacteria. This process requires molecular oxygen and TPNH, and generates a double bond only at the ninth carbon position.

b. Anaerobes, facultative aerobes, and some obligate aerobic bacteria lack the ability to desaturate stearic and palmitic acids, either in the absence or presence of oxygen. In these pathways, olefinic acids (Δ^7-C_{16}, Δ^9-C_{16}, Δ^9-C_{18}, and Δ^{11}-C_{18}) are synthesized in the course of a chain-lengthening process rather than by desaturation of the corresponding saturated acids. This nonoxidative pathway appears to be employed not only by the obligate anaerobes, but also by various aerobic representatives. The anaerobic biosynthetic routes to monounsaturated fatty acids in bacteria are postulated. The fatty acids which have been isolated from bacterial lipids are underlined. The unsaturated sites of these underlined acids are not only at the carbon, but also at the seventh and eleventh carbon.



c. Multiply-unsaturated fatty acids are formed from oleic acid or palmitoleic acid by an oxidative mechanism. They are not synthesized or found in microorganisms in which vaccenic acid (Δ^{11} -C_{18}) is the principal C_{18} unsaturated fatty acid, nor is there evidence that they occur in any bacteria lipids.

The composition of fatty acids in microorganisms are highly dependent upon the growing conditions. Milner[27] has reported that in Chlorella pyrenoidosa grown either in a greenhouse or outside, the amounts of C_{16} and C_{18} acids were inverted and the amount of C_{16} saturated and unsaturated fatty acids decreased with increasing lipid content of the cell. Nichols[28] has shown that the amount of linolenic acid in Chlorella vulgaris is much greater when it is grown on a mineral medium supplemented with amino acids, under light than when it is grown in an organic medium and in the dark. Hexadecatrienoic acid is essentially absent except on an inorganic medium under light.

From the lipids of B. subtilis, Saito[16] and Kaneda[17] isolated 13-methyltetradecanoic, 14-methylpentadecanoic, 15-methylhexadecanoic, and 14-methylhexadecanoic acids. Some of these acids as well as 12-methyltetradecanoic acid have been identified in the membranes of B. megaterium,[18] streptomyces sp.,[19] Sarcina species,[22] and St. Aureus.[21]

Iso- and anteiso-fatty acids are synthesized by condensation of short chain iso- or anteiso-acid coenzyme A esters with malonylcoenzyme A in the presence of TPNH.[22-24] Oró[25] reported uniformly labeled L-isoleucine-14C was predominantly incorporated into the anteiso-branched chain acids in Sarcina lutea. Bennet[26] found that labeled a-butyric acid was incorporated mainly in anteisoheptadecanoic acid, and labeled isobutyrate gave rise to isotetradecanoic and isohexadecanoic acids.

Cyclopropane fatty acids were found by Hofmann et al.[29] and Law et al.[30] The biosynthesis of lactobacillic acid and other cyclopropane fatty acid has been studied extensively.[31-33] By using either labeled cis-vaccenic acid or CH_3-^{14}C-methionine, it was shown that lactobacillic acid was formed by addition of a one-carbon unit to the double bond of cis-vaccenic acid. A similar pathway existed in M. phle, the methionine supplied the methyl side chain to oleic acid for the formation of tuberculostearic acid (10-methyloctadecanoic acid).[34] Cason and Miller[35] isolated 10-methylhexadec-9-enoic acid in addition to 10-methylhexadecanoic acid from the lipids of M. tuberculosis. It is therefore feasible that the addition of the methyl group of methionine to the double bond of a monounsaturated precursor proceeds via an unsaturated intermediate.

Most of the hydroxy acids isolated from microorganisms which are β-hydroxy acids. In all β-hydroxy acids the steric configuration of the asymmetric carbon belong to the D-series. Kaneshiro[36] characterized 3-hydroxydodecanoic acid and along with 2-hydroxy isomer from Azotobacter agilis. Bishop[37] observed 1.5% of 3-hydroxydodec-5-enoic acid in Lactobacilli, which could be explained as a precursor of cis-vaccenic acid. Polgar and Smith[38] found 10-hydroxyoctadecanoic acid and a keto acid, 10-oxooctadecanoic acid from the lipids of human strains of M. tuberculosis.

3. Fatty Acids in Higher Plants and Animals

The lipids from different parts of the same plant, e.g., seeds, leaves, and roots, may have a different fatty acid composition as well as different types of fatty acids. In seeds, moreover, the lipids are largely triglycerides,

whereas in the leaves triglycerides are replaced by galactolipids. In the brain of animals phosphoglycerides are the dominant compositions. In bacteria the lipids consist largely of waxes together with lesser amounts of so-called acetone soluble "fats".

Linolenic acid is usually a major or chief fatty acid constituent in leaf lipids. Crombie's[39] results show that the lipids of green as compared with those of non-green leaves contain much more linolenic but less linoleic and oleic acids, and generally confirm the high levels of linolenic acid in green leaves. Debuch[40] has isolated hexadeca-*trans*-3-enoic acid as well as hexadeca-*cis*-10, *cis*-13-trienoic acid in the leaf lipids of *Spinacea oleracea* and of *Antirrhinum majus*. This hexadecatrienoic acid was first reported by Heyes[41] in 1951 as a major constituent of the acetone-soluble lipids of *Brassica napus* leaves.

The early work[42,43] showed that when rats and other species of animals were fed diets from which fat was excluded, growth and reproduction were impaired and survival was limited. A dermatitis, characterized by a scaliness of the tail and paws, soon developed. All these symptoms could be reversed by the addition of polyunsaturated fatty acids to the diet. These fatty acids are linoleic, linolenic, and arachidonic acid. These fatty acids are called essential fatty acids or vitamin F. Since polyunsaturated fatty acids are required by the animal, presumably animal is unable to synthesize them from other materials, and it must obtain these acids from the food.

It is now recognized that many animal species require essential fatty acids for proper nutrition, *e.g.*, rats, mice, guinea pigs, hamsters, dogs,

calves, as well as human infant, etc. Apparently, however, either the young animal is able to store essential fatty acids for future use, or the requirement for these essential fatty acids is markedly decreased in older animals. Since it is practically impossible to produce an essential fatty acid-deficiency syndrome in the adult of any species, the possibility does exist, however, that the utilization of essential fatty acids may be impaired in certain disease conditions which could increase the requirement for the adult animal.

Essential fatty acids occur naturally and in considerable quantity, but not exclusively, in vegetable and seed oils. Cottonseed oil, corn oil and soybean oil contain approximately 50% linoleic acid as the triglyceride; walnut oil, safflower oil, and wheat germ oil are considerably higher in linoleic acid content. The vegetable oil, olive oil, is rather low in linoleic acid (10%). On the other hand, beef tallow and most of animal greases contain less than 10% of total polyunsaturated fatty acids, 40 to 60% of oleic acid, and the saturated fatty acids contents are rather higher. In general, green leaves have a much higher polyunsaturated fatty acid content of linolenic acid and arachidonic acid, but animal grease and animal tallow are richer in saturated fatty acids.

4. Sample and Experimental

The samples studied for fatty acids have been described in Chapter 6. Extraction, fractionation, separation, methylation, as well as gas chromatographic and mass spectrometric methods used here, which could be found in Chapter 2.

Two 100-ft. 0.01 inch inside diameter capillary columns coated with Apiezon L and HI-EFF-4BP, were used for separation, as well as coinjection

studies. Acids were converted to methyl esters before the final separation and identification. All components in Tables I, II, and III were identified by both fragmentation patterns of mass spectra and coinjections on two capillary columns. The unsaturated fatty acid methyl esters have a longer retention time than the same carbon numbered saturated isomers in polyester column (HI-EFF-4BP), but they come out faster than saturated ones on Apiezon column.

Authentic acids were purchased from Applied Science Lab., Aldrich Chemical Company, K. and K., and Calbiochem. The cyclopropyl-C_{17} (methylene-9,10-hexadecenoic acid) and cyclopropyl-C_{19} (methylene-11,12-octadecenoic acid) were synthesized in this laboratory. The following procedures are used for making these two cyclopropyl compounds.

The general reaction for the preparation of cyclopropanes from olefins, diiodomethane, and zinc-copper couple was developed by Simmons and Smith,[44] and was modified by LeGoff.[45] The granular zinc was Baker's 30-mesh reagent grade. This granular zinc (30 g) was added to a hot solution of 0.5 g of cupric acetate monohydrate in 50 ml of glacial acetic acid. The mixture was shaken for 3 minutes, keeping it hot during this period to prevent precipitation of zinc acetate. After the acetic acid was decanted, the zinc-copper couple was washed with a 50-ml portion of glacial acetic acid, and then with three portions of ether. This zinc-copper couple was used for the preparation of methylene-9,10-hexadecenoic acid and methylene-11,12-octadecenoic acid.

The solvent, 50 ml of ether, was added to 10 g of zinc-copper couple, followed by 2 g of the diiodomethane. The stirred suspension was kept at gentle reflux. A mixture of 15.5 g (0.05 mole) of methyl vacenate and 2.4 g of diiodomethane was added dropwise over a period of 20 min. The reaction mixture was stirred overnight. The ether solution was then slowly decanted

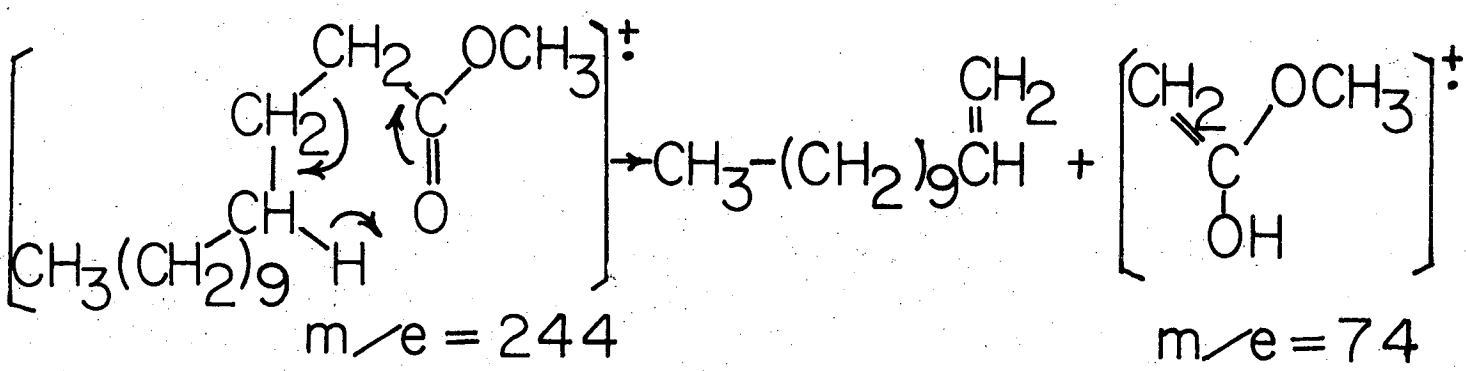
from the unchanged couple into a separatory funnel containing a mixture of ice and 1 N HCl. The ether solution was separated, washed with ice-HCl again, washed three times with water, and finally dried over potassium carbonate. After the solvent was removed by a rotary evaporator, 7.5 g (45.8% yield) of the final product was obtained.

The same procedure was used to synthesize cyclopropyl-C_{17} methyl ester. About 40% yield was obtained from this synthesis.

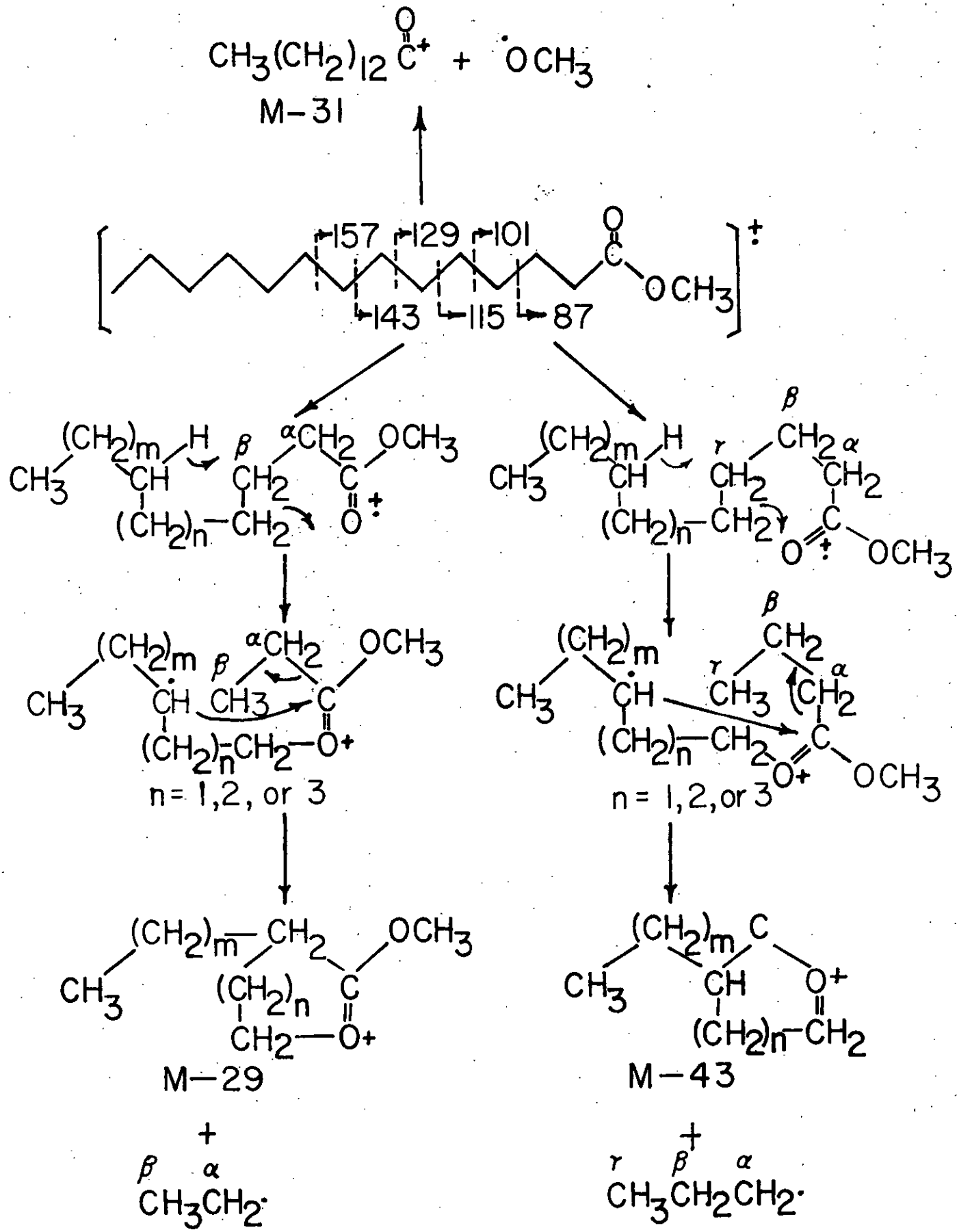
5. Mass Spectrometric Identification

Figs. 1 and 2 show part of the mass spectra which are taken by combined gas chromatographic-mass spectrometric analysis. Fig. 1 shows the mass spectra of methyl myristate, methyl linolenate, and methyl oleate.

The n-C_{14} methyl ester represents the straight chain saturated ester. This type of esters has a base peak at m/e= 74 in spectra. It is produced by McLafferty rearrangement involving the γ-hydrogen migration to the carboxyl oxygen of the ester group with a concerted β-cleavage.[46] This results in a neutral olefin molecule and a charged enol.



The next intense peak is m/e =87 in Fig. 1 n-C_{14} spectrum, which is formed by simple β-cleavage of the chain.


CH3(CH2)12 C+ + OCH3
M-31
157
129
101
143
115
87
OCH3
n = 1,2, or 3
n = 1,2,or 3
M-29
M-43

Some other intense peaks, m/e 101, 129, 143, 157, are also formed by simple cleavage and charges stay on the oxygen containing fragments. The fragment at m/e 143 which gives a relatively high intensity in the mass spectra of saturated long chain methyl esters, is originated through a single bond cleavage between the 7th and 8th carbon. Beynon[47] suggested a rearranged dicyclic structure for this cleavage. The M-31 peak which is formed by splitting out a methoxyl group from the saturated long chain methyl ester.

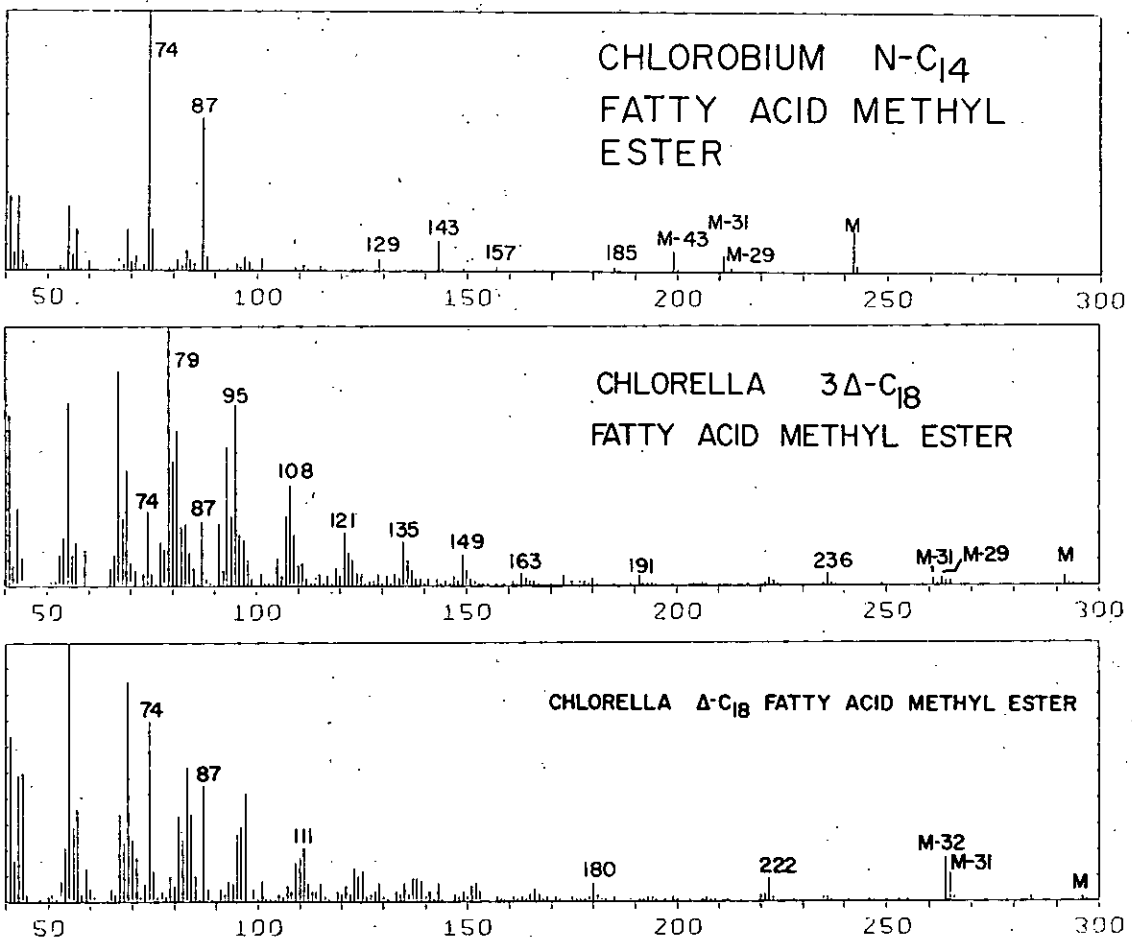
Ryhage et al.[48] used deuterium labeling experiments to study the M-43 and M-29 fragmentation pathways. These two peaks originate through an expulsion mechanism rather than by loss of the terminal propyl and ethyl groups. The expulsion of α and β carbon atoms gives rise to the M-29 peak, while the M-43 peak is formed by expulsion of α, β, and γ carbon atoms.

Mass spectra showing the M-32, M-74, and M-116 peaks were observed for the methyl oleate (Fig. 1), methyl vaccenate, and other mono-unsaturated fatty acid methyl esters. The mass spectra of double bond positional isomers of monounsaturated esters are very much alike. The M-32 peak corresponds to the loss of methanol. The peak at M-74 is produced by β cleavage with concerted rearrangement of a γ-hydrogen atom to the carbonyl oxygen of the ester group.[46,49] This is the same way in which the base peak of a saturated methyl ester is produced. The only difference is that the double bond supplies a π-orbital, where the charge could stay on the non-oxygen contained fragment. The peak at M-116 arises from 5,6-carbon bond cleavage. Slightly different characteristic fragmentation patterns were found with the di- and trienoic fatty acid methyl esters (Fig. 1). Molecular ions are the major indications for the

polyunsaturated esters. The mass spectrometric fragmentation patterns of cyclopropyl-C_{17} and cyclopropyl-C_{19} acid methyl esters are found to be very similar to the corresponding monounsaturated isomers (Fig. 2). Few studies have been done on this type of ester.

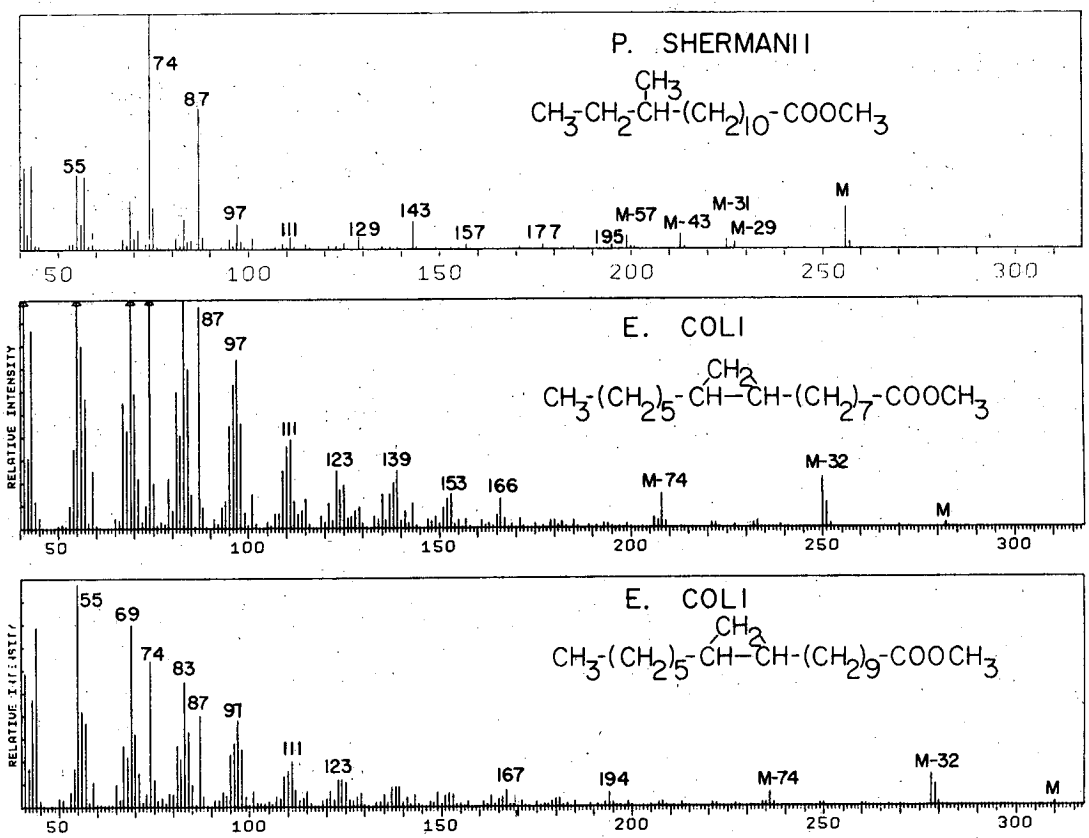
The iso- and anteiso-acid methyl esters could be identified by their mass spectrometric fragmentation patterns. The bonds next to the methyl branched site would be broken, so that the loss of methyl and isopropyl groups are found in the spectra of iso-isomers while anteiso-isomers will split out ethyl and isobutyl groups (Fig. 2, M-29, M-43).

Figure 1: Mass spectra of methyl myristate, methyl linolenate, and methyl oleate from Chlorobium and Chlorella. Obtained using a combination of an Aerograph 204 gas chromatograph and an A.E.I. MS-12 mass spectrometer.


CHLOROBIUM N-C14
FATTY ACID METHYL
ESTER
74
87
129
143
157
185
M-43
M-31
M-29
M
50
100
150
200
250
300
CHLORELLA 3Δ-C18
FATTY ACID METHYL ESTER
79
95
108
74
87
121
135
149
163
191
236
M-31
M-29
M
50
100
150
200
250
300
CHLORELLA Δ-C18 FATTY ACID METHYL ESTER
74
87
111
180
222
M-32
M-31
M
50
100
150
200
250
300

XBL 670-6231

Figure 2: Mass spectra of methyl esters of anteiso-C_{15}, cyclopropyl-C_{17}, and cyclopropyl-C_{19} acids from P. Shermanii and E. Coli. Obtained using a combination of an Aerograph 204 gas chromatograph and an A.E.I. MS-12 mass spectrometer.


P. SHERMANII
CH3-CH2-CH(CH3)-(CH2)10-COOCH3
74
87
55
97
111
129
143
157
177
195
M-57
M-43
M-31
M-29
M
50
100
150
200
250
300
E. COLI
CH3-(CH2)5-CH(CH2)-CH-(CH2)7-COOCH3
RELATIVE INTENSITY
87
97
111
123
139
153
166
M-74
M-32
M
50
100
150
200
250
300
E. COLI
CH3-(CH2)5-CH(CH2)-CH-(CH2)9-COOCH3
55
69
74
83
87
97
111
123
167
194
M-74
M-32
M
50
100
150
200
250
300

XBL 703-5074

The best clue to the position of the methyl branch is obtained from the presence of ketene ion (m/e 195)[50] which is derived from the branched structure through the loss of a terminal alkyl and methanol (Fig. 2). The loss of water from the ketene ion gives a peak m/e 177 (Fig. 2). Although both peaks are present as very low intensities, they are reliable since they are absent in the spectra of normal saturated and unsaturated fatty acids. The same two fragments, the ketene ion and the peak which loss of water from ketene, are also found in the spectra of iso acid methyl esters. Thus, these two ions could be used as important information to interpret the spectra of methyl branched fatty esters.

6. Results and Discussion

Fatty acid constituents of total lipid of algae are shown in Table I. All acids identified by gas chromatography and their mass spectra are even carbon numbered fatty acids range from C_{12} to C_{18}. The algae contain an undetectable amount of branched-chain fatty acids, which confirms the report by Parker.[51]

The presence of polyunsaturated fatty acids are rather low in *Anacystis nidulans* (Fig. 3A), but their compositions are higher in the other three blue-green algae (Fig. 3B,C). The green algae, *Chlorella pyrenoidosa*, contain more than 50% of 3Δ-C_{16} and 3Δ-C_{18} (Fig. 3D). Since polyunsaturated fatty acids are formed under light by oxidation and dehydration mechanisms, so it is reasonable to assume green algae have a higher oxidative power than blue-green algae. *Trichodesmium*, a blue-green algae,[51] contains 50% of n-C_{10} acid; this acid is not found in those four species which have been examined. Algae with this high concentration of acids of low molecular weight are rare.

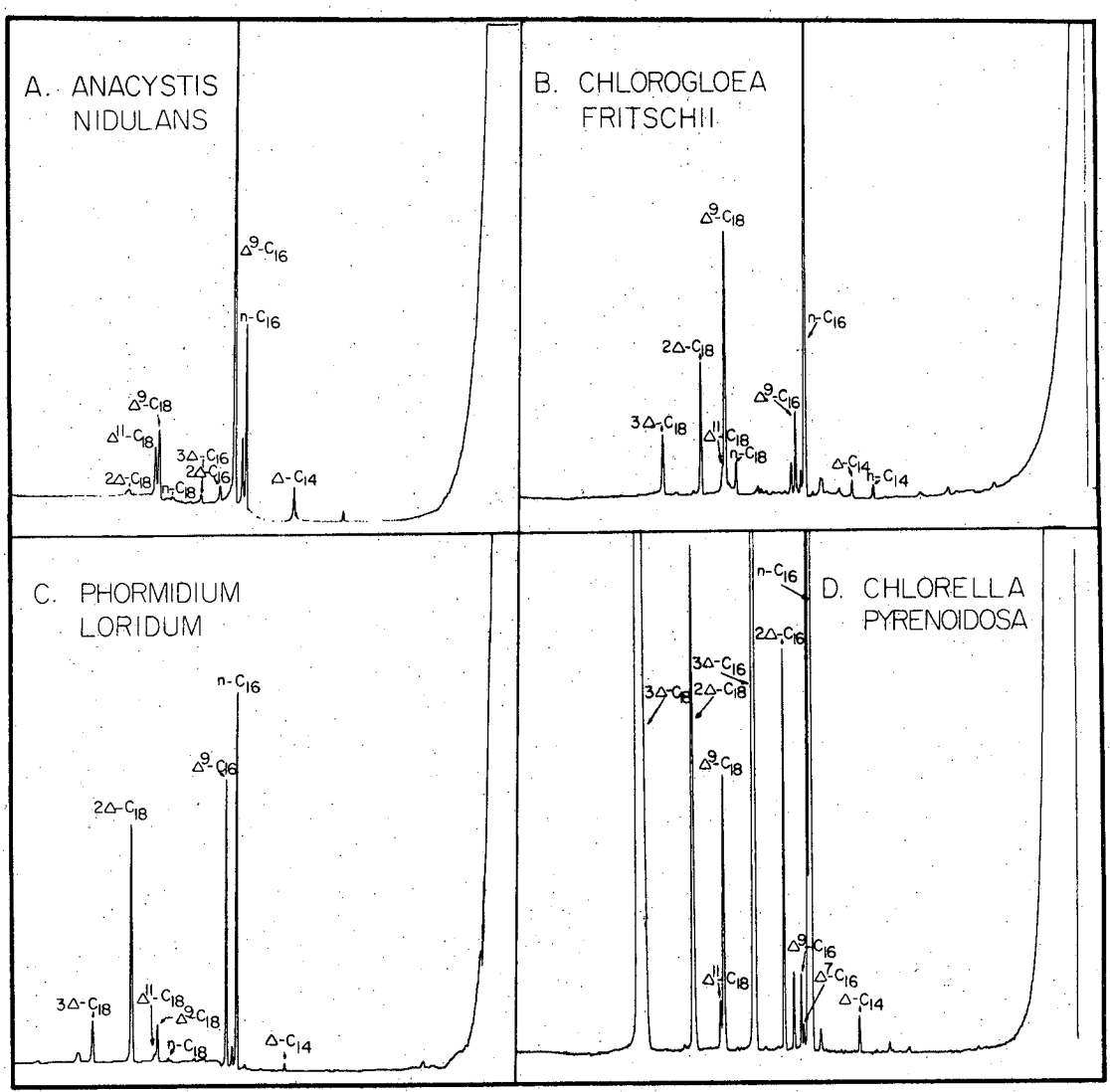
Table I

FATTY ACIDS COMPOSITION OF TOTAL LIPIDS OF ALGAE

	Blue-green Algae				Green Algae
	Anacystis nidulans	Nostoc muscorum	Phormidium loridum	Chlorogloea fritschii	Chlorella pyrenoidosa
n-C_{14}	--	--	0.2	2.0	--
Δ-C_{14}	2.8	--	0.4	2.1	0.9
n-C_{16}	12.5	23.4	35.8	45.7	18.9
Δ^{7}-C_{16}	--	--	--	--	0.5
Δ^{9}-C_{16}	61.0	29.8	23.9	5.6	0.9
Δ^{11}-C_{16}	--	--	--	--	--
2Δ-C_{16}	1.8	1.5	--	--	4.2
3Δ-C_{16}	1.0	--	--	--	19.7
n-C_{17}	--	--	--	--	--
Δ-C_{17}	--	--	--	--	--
n-C_{18}	0.3	0.6	0.7	3.1	--
Δ^{9}-C_{18}	4.8	13.8	4.8	20.0	3.8
Δ^{11}-C_{18}	3.4	1.2	1.4	2.2	0.2
2Δ-C_{18}	0.1	18.3	21.5	10.5	6.8
3Δ-C_{18}	--	8.6	8.4	4.9	45.5
n-C_{19}	--	--	--	--	--
Δ-C_{19}	--	--	--	--	--

Figure 3: Gas chromatographic separation of total acid fractions using Aerograph-204 instrument. A stainless steel tubing 100 ft. x 0.01 in., coated with HI-EFF-4BP. Helium pressure 20psi; no split. Temperature was programmed at $2^{o}C$/min. from $90^{o}C$ to $200^{o}C$.

A. Total fatty acids from Anacystis Nidulans
B. Total fatty acids from Chlorogloea Fritschii
C. Total fatty acids from Phormidium Loridum
D. Total fatty acids from Chlorella Pyrenoidosa


A. ANACYSTIS NIDULANS
Δ9-C16
n-C16
Δ9-C18
Δ11-C18
3Δ-C16
2Δ-C16
2Δ-C18
n-C18
Δ-C14
B. CHLOROGLOEA FRITSCHII
Δ9-C18
n-C16
2Δ-C18
Δ9-C16
3Δ-C18
Δ11-C18
n-C18
Δ-C14
n-C14
C. PHORMIDIUM LORIDUM
n-C16
Δ9-C16
2Δ-C18
3Δ-C18
Δ11-C18
Δ9-C18
n-C18
Δ-C14
D. CHLORELLA PYRENOIDOSA
n-C16
2Δ-C16
3Δ-C16
3Δ-C18
2Δ-C18
Δ9-C18
Δ9-C16
Δ7-C16
Δ11-C18
Δ-C14

XBL 703-5065

Double-bond position isomers have been resolved by capillary column gas chromatography. This separation has not been done before. By the aid of this technique, oleic acid (Δ^9-octadecenoic acid) and vaccenic acid (Δ^{11}-octadecenoic acid) are found in all of the algae. The ratio of these two acids is 1.4 to 11.5 in favor of oleic acid. Oleic acid is formed either by desaturation of steric acid under oxygen or by chain lengthening process from small unsaturated acids. Vaccenic acid has to be formed by the latter mechanism. Palmitoleic acid (Δ^9-hexadecenoic acid) is detected as the sole monounsaturated C_{16} acid in blue-green algae; however, both Δ^9- and Δ^7-hexadecenoic acid are found in green algae (Fig. 3). Palmitic acid is one of the major peaks in all the algae species, but saturated C_{12}, C_{14}, and C_{18} acids are the minors that confirm the other group's work.[52-54]

Four species of photosynthetic bacteria have been analyzed. Table II shows their total fatty acid composition. In contrast to algae, saturated-C_{17} acid is detected in two cases--*Rhodospirillum rubrum* and *Rhodomicrobium vanniellii*. Palmitic acid is no more one of the most abundant components in the total fatty acid mixtures. Chlorobium, a sulfur anaerobic photosynthetic bacteria, contains 27.6% of myristic acid (n-C_{14}) which is considerably higher in photosynthetic microorganisms. Two cyclopropyl acids, cyclopropyl-C_{17} (9, 10-methylenehexadecanoic acid) and cyclopropyl-C_{19} (11,12-methyloctadecanoic acid) are found in some of the photosynthetic bacteria. They are formed by the fixation of a methylene group derived from methionine to *cis* Δ-C_{18} and Δ-C_{16} acids.[31-33] Polyunsaturated acids are absent except chloro-

Table II

FATTY ACIDS COMPOSITION OF TOTAL LIPIDS OF PHOTOSYNTHETIC BACTERIA

	Aerobic Bacteria		Anaerobic Bacteria	
	Rhodopseudomonas spheroides	Rhodospirillum rubrum	Chlorobium (sulfur bacteria)	Rhodomicrobium vannielii
n-C_{14}	--	0.5	27.6	0.4
Δ-C_{14}	0.5	--	1.4	--
n-C_{16}	4.7	21.9	10.9	3.1
Δ^{7}-C_{16}	--	--	--	--
Δ^{9}-C_{16}	3.8	32.1	31.2	0.7
Δ^{11}-C_{16}	--	--	--	--
2Δ-C_{16}	--	--	0.8	--
3Δ-C_{16}	--	--	--	--
n-C_{17}	--	0.7	--	0.2
cyclopropyl-C_{17}	--	--	21.5	0.1
n-C_{18}	0.3	0.5	2.1	1.3
Δ^{9}-C_{18}	--	--	--	--
Δ^{11}-C_{18}	87.4	41.5	--	92.4
2Δ-C_{18}	--	--	--	--
3Δ-C_{18}	--	--	--	--
n-C_{19}	--	--	--	--
cyclopropyl-C_{19}	--	0.6	--	0.6
Δ-C_{20}	--	--	--	0.6

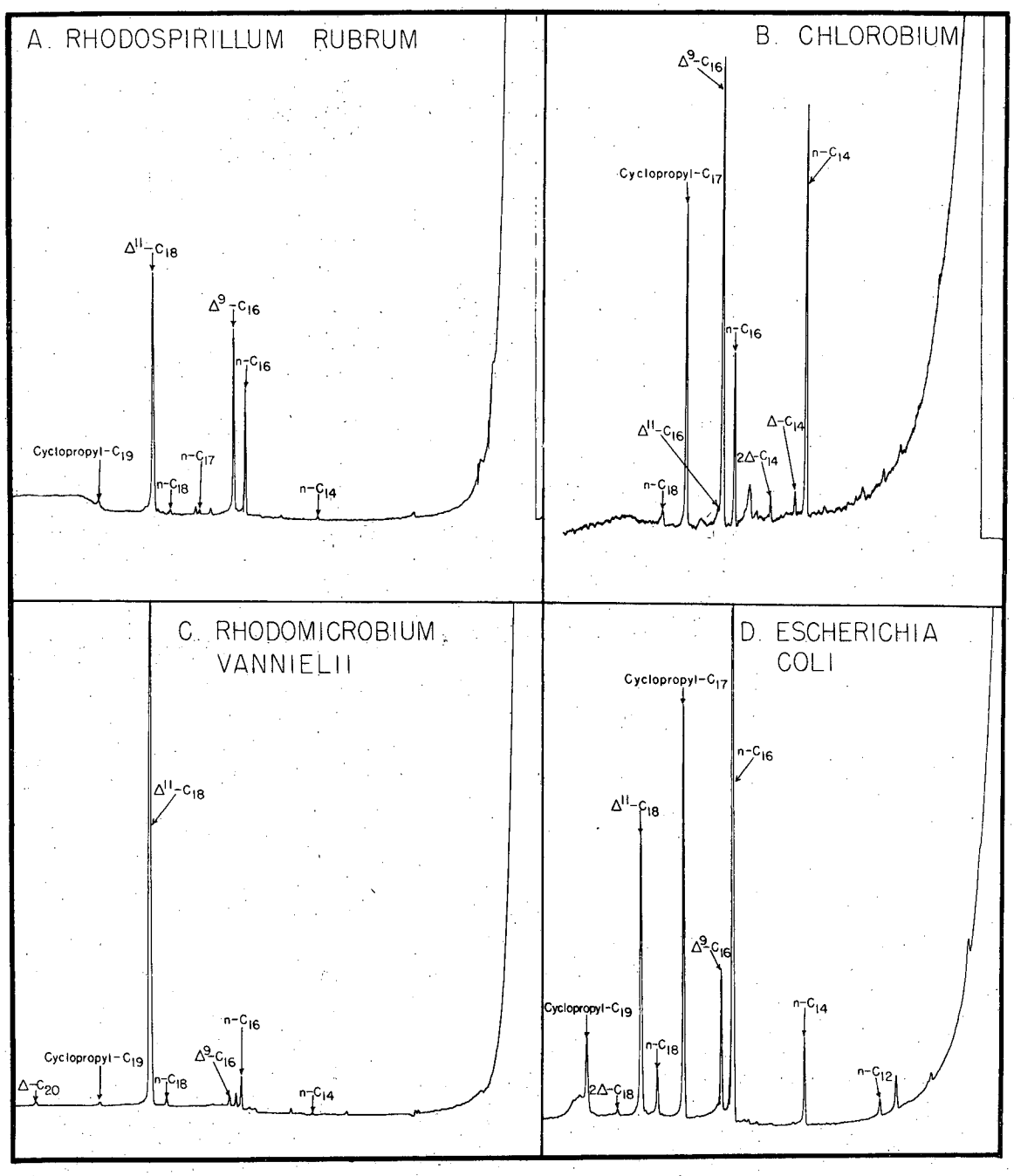
Figure 4: Gas Chromatographic separation of total fatty acids. All the conditions are the same as Figure 3.

A. Total fatty acids from Rhodospirillum Rubrum

B. Total fatty acids from B. Chlorobium

C. Total fatty acids from Rhodomicrobium Vannielii

D. Total fatty acids from Escherichia Coli


A. RHODOSPIRILLUM RUBRUM
Δ11-C18
Δ9-C16
n-C16
Cyclopropyl-C19
n-C17
n-C18
n-C14
B. CHLOROBIUM
Δ9-C16
n-C14
Cyclopropyl-C17
n-C16
Δ11-C16
Δ-C14
2Δ-C14
n-C18
C. RHODOMICROBIUM VANNIELII
Δ11-C18
n-C16
Cyclopropyl-C19
Δ9-C16
Δ-C20
n-C18
n-C14
D. ESCHERICHIA COLI
Cyclopropyl-C17
n-C16
Δ11-C18
Δ9-C16
Cyclopropyl-C19
n-C14
n-C18
2Δ-C18
n-C12

XBL 703-5063

bium, which contains small amounts of 2Δ-C_{16} and 2Δ-C_{14}. This evidence could indicate that the oxidation potential in bacteria is smaller than blue-green algae and much smaller than green algae. Monounsaturated fatty acids found in all four species are palmitoleic and vaccenic acids. Since oleic acid is absent in photosynthetic bacteria, one may consider both Δ^9-C_{16} and Δ^{11}-C_{18} acids are formed by anaerobic chain-elongation mechanism. Rhodopseudomonas spheroides and Rhodomicrobium vannielii contain 87.4 and 92.4% of vaccenic acid respectively. Wood, Nichols, and James[55] have shown the major fatty acids of Rhodospirillum rubrum and four species of Rhodopseudomonas which produced under both anaerobic light and aerobic dark conditions. The major components in all cases are the C_{16} and C_{18} carbon straight chain and monoenoic fatty acids. Changing from light to dark growth usually causes a drop in the amount of saturated acid and an increase in C_{16} monoene. Their results show that vaccenic acid in Rhodopseudomonas spheroides increased from 91% under light and anaerobic to 99% under dark aerobic growth.

Escherichia coli is the only aerobic non-photosynthetic bacteria examined in this laboratory (Table III), which contains even-numbered normal saturated fatty acid from C_{12} to C_{18} with palmitic acid predominant (Fig. 4D). The presence of cyclopropyl-C_{17} and cyclopropyl-C_{19} acids in E. coli from anaerobically grown, as reported by Bloch et al.[56] are confirmed and extended to aerobic grown. The unsaturated acids found in E. coli are palmitoleic and vaccenic acids with small amount of 2Δ-C_{18}.

Micrococcus lysodeikticus, P. shermanii, two species of Clostridium, and three strains of Desulfovibrio are studied for their fatty acid content.

Table III

FATTY ACIDS COMPOSITION OF TOTAL LIPIDS OF NON-PHOTOSYNTHETIC BACTERIA

	Aerobic Bacteria		Anaerobic Bacteria		
Saturated Acids	Escherichia coli	Micrococcus lysodeikticus	Clostridium acidiurici	Clostridium tetanomorphum	P.shermanii
n-C_{12}	0.8	--	0.8	0.3	0.4
iso-C_{13}	--	--	--	--	0.2
anteiso-C_{13}	--	--	--	--	1.6
n-C_{13}	--	--	--	--	--
iso-C_{14}	--	--	--	--	0.4
anteiso-C_{14}	--	--	--	--	0.1
n-C_{14}	4.8	12.6	54.5	85.0	0.2
iso-C_{15}	--	--	--	--	3.3
anteiso-C_{15}	--	--	--	--	84.5
n-C_{15}	--	--	--	0.3	1.3
iso-C_{16}	--	--	--	--	0.1
anteiso-C_{16}	--	--	--	--	0.3
n-C_{16}	36.8	32.2	3.3	4.2	0.6
iso-C_{17}	--	--	--	--	0.4
anteiso-C_{17}	--	--	--	--	2.2
n-C_{17}	--	--	--	--	2.6
iso-C_{18}	--	--	--	--	0.2
anteiso-C_{18}	--	--	--	--	--
n-C_{18}	2.4	10.5	0.2	0.2	0.2
iso-C_{19}	--	--	--	--	0.1
anteiso-C_{19}	--	--	--	--	0.1
n-C_{19}	--	--	--	--	0.3
iso-C_{20}	--	--	--	--	--
anteiso-C_{20}	--	--	--	--	--
n-C_{20}	--	--	--	--	--
n-C_{21}	--	--	--	--	--
Cyclopropane C_{17}	22.9	--	--	--	--
Cyclopropane C_{19}	4.2	--	--	--	--

Table III (cont'd)

Unsaturated Acids	Aerobic Bacteria: Escherichia coli	Aerobic Bacteria: Micrococcus lysodeikticus	Anaerobic Bacteria: Clostridium acidiurici	Anaerobic Bacteria: Clostridium tetanomorphum	Anaerobic Bacteria: P.shermanii
Δ-C_{14}	--	1.9	5.1	0.8	--
2Δ-C_{14}		14.2	--	--	--
Δ^{7}-C_{16}	--	3.9	0.3	1.7	--
Δ^{9}-C_{16}	7.7	1.3	34.3	4.5	--
Δ^{11}-C_{16}	--	--	0.3	0.4	--
Δ^{9}-C_{18}	--	15.5	--	--	--
Δ^{11}-C_{18}	15.8	5.8	--	--	--
2Δ-C_{18}	0.4	1.0	--	--	--

Saturated Acids	Anaerobic Bacteria: Desulfovibrio vulgaris strain "Hildenborough"	Anaerobic Bacteria: Strain "Essex 6"	Anaerobic Bacteria: Strain "Berre Sol"	Anaerobic Bacteria: Yeast
n-C_{12}	--	--	--	--
iso-C_{13}	--	--	--	--
anteiso-C_{13}	--	--	--	--
n-C_{13}	--	--	--	--
n-C_{14}	1.1	1.2	2.6	0.8
iso-C_{15}	29.2	7.4	12.5	--
anteiso-C_{15}	2.2	0.8	0.9	--
n-C_{15}	--	0.6	--	--
n-C_{16}	14.6	41	41	19.4
iso-C_{17}	13.1	3.0	0.9	--
anteiso-C_{17}	1.2	0.1	0.2	--
n-C_{17}	--	0.6	--	--
n-C_{18}	9.6	4.5	3.9	4.2
iso-C_{19}	--	0.2	--	--
anteiso-C_{19}	--	0.3	--	--
n-C_{19}	0.2	0.6	--	--
n-C_{20}	--	7.4	--	--
n-C_{21}	--	1.0	--	--
Cyclopropane C_{17}	--	--	--	--
Cyclopropane C_{19}	--	--	--	--

Table III (cont'd)

Unsaturated Acids	Anaerobic Bacteria: Desulfovibrio vulgaris strain "Hilden-borough"	Strain "Essex 6"	Strain "Berre Sol"	Yeast
Δ-C_{14}	--	--	--	--
2Δ-C_{14}	--	--	--	--
Δ^{7}-C_{16}	0.3	1.2	--	--
Δ^{9}-C_{16}	1.0	7.6	.6	57.6
Δ^{11}-C_{16}	--	--	--	--
Δ^{9}-C_{18}	1.6	6.4	0.9	16.6
Δ -C_{18}	2.1	1.2	6.6	0.8
2Δ-C_{18}	--	--	--	--

These groups of microorganisms are anaerobic non-photosynthetic bacteria. In two species of Clostridium which contain more than 50% of myristic acid in the total acids fractions, but other anaerobes are richer in palmitic acid. Normal C_{17} saturated acid is present in some of the anaerobic bacteria. Cyclopropyl-C_{17} and cyclopropyl-C_{19} acids are not found in these species. Unsaturated fatty acids are absent in P. shermanii (Fig. 5B) but present in other species in small amounts. A small peak in Clostridium next to Δ^9-C_{16} acid but it has a longer retention time than Δ^9-C_{16} on gas chromatography (Fig. 5C & D). Its mass spectrum is identical to Δ^7-C_{16} and Δ^9-C_{16} methyl esters. Since there is no authorized sample on hand, it is tentatively identified as Δ^{11}-C_{16} acid, which could be the first finding of this isomer existing in living organism. A series of iso- and anteiso-fatty acids are detected by gas chromatography and mass spectrometry. These iso- and anteiso-acids present in all three strains of Desulfovibrio are all odd carbon numbered acids (Fig. 6A, B & C). The ratio of iso- to anteiso-compounds is greater than 10 in favor of iso-acids. P. shermanii contains a small amount of even-numbered iso- and anteiso-acids also. In contrast to Desulfovibrio, P. shermanii has much more anteiso- than iso-acids for the odd-numbered branched acids. However, the ratio of iso- to anteiso-acid is greater than one for even-numbered acids in P. shermanii. In an anaerobic bacteria, if iso- to anteiso- acids ratio is greater than one for the odd acids, then the ratio has to be smaller than one for the even-numbered isomers, or vice versa. By examining other groups' reports,[57-61] the fact is true indeed.

Yeast contains normal fatty acids from C_{14} to C_{18} and palmitoleic, oleic, and viccenic acid. More comprehensive studies of the fatty acid constituent in yeast are being carried on by Peters in this laboratory.

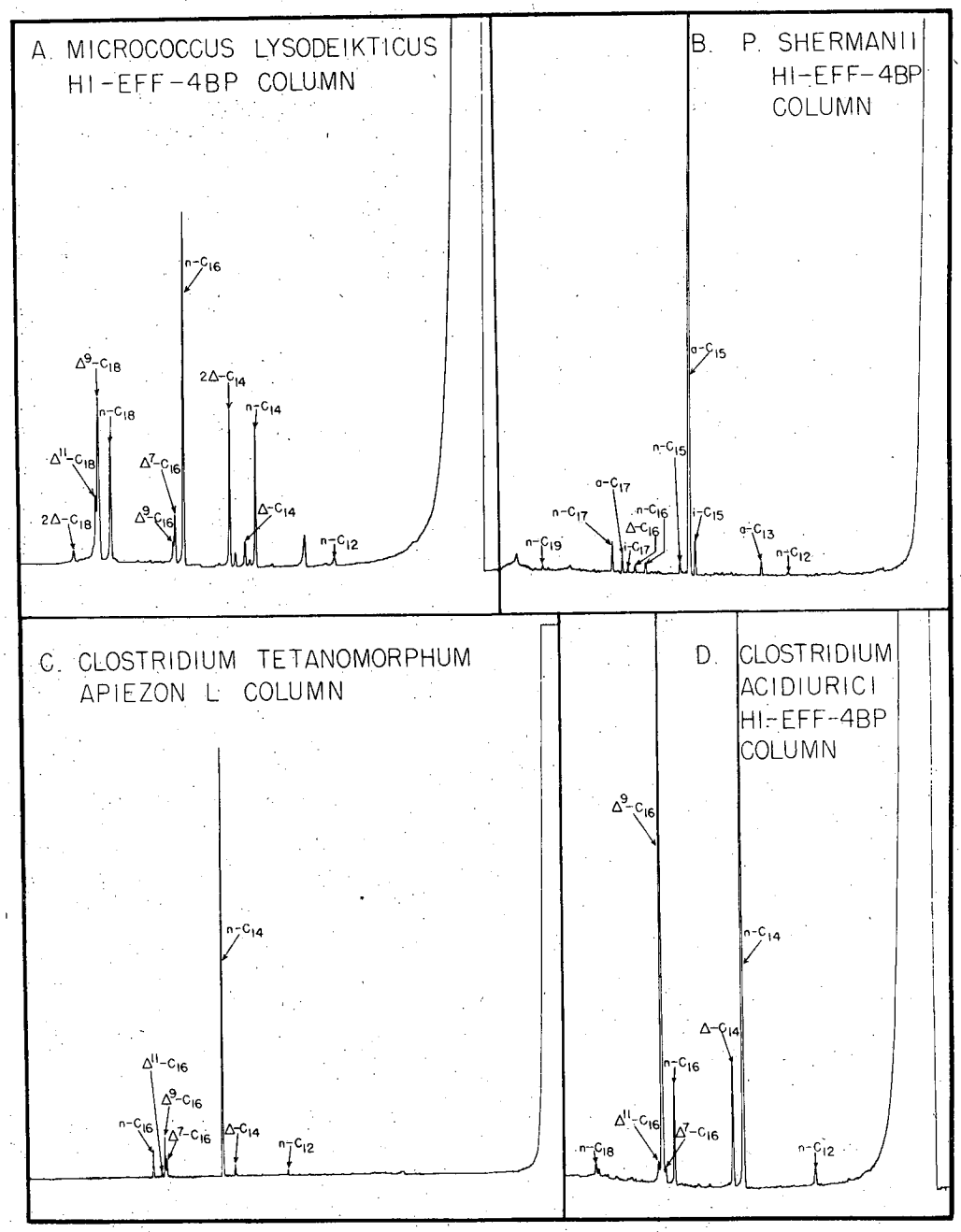
Figure 5: Gas chromatographic separation of total fatty acid fractions. Stainless steel columns 100 ft. x 0.01 in., coated with Apiezon L and HI-EFF-4BP were used. Other conditions are the same as Figure 3.

A. Total fatty acids from Microceccus Lysodeikticus

B. Total fatty acids from P. Shermanii

C. Total fatty acids from Clostridium Tetanomorphum

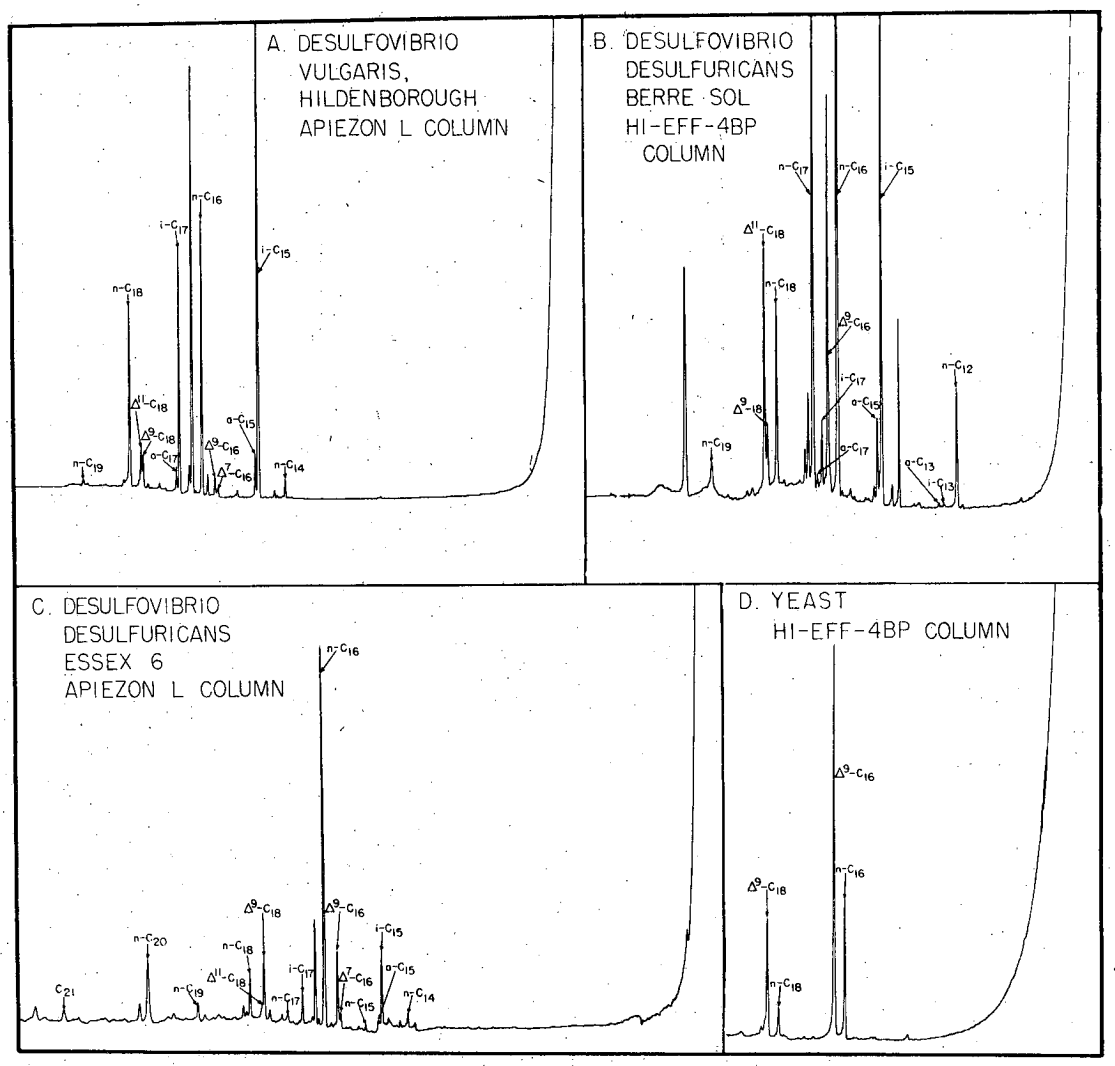
D. Total fatty acids from Clostridium Acidi Urici


A. MICROCOCCUS LYSODEIKTICUS
HI-EFF-4BP COLUMN
n-C16
Δ9-C18
n-C18
Δ11-C18
2Δ-C18
Δ7-C16
Δ9-C16
2Δ-C14
n-C14
Δ-C14
n-C12
B. P. SHERMANII
HI-EFF-4BP
COLUMN
a-C15
n-C15
a-C17
n-C17
n-C16
Δ-C16
i-C17
n-C19
i-C15
a-C13
n-C12
C. CLOSTRIDIUM TETANOMORPHUM
APIEZON L COLUMN
n-C14
Δ11-C16
Δ9-C16
n-C16
Δ7-C16
Δ-C14
n-C12
D. CLOSTRIDIUM
ACIDIURICI
HI-EFF-4BP
COLUMN
Δ9-C16
n-C14
Δ-C14
n-C16
Δ11-C16
Δ7-C16
n-C18
n-C12

XBL 703-5064

Figure 6: Gas chromatographic separation of total fatty acid fractions. Stainless steel columns 100 ft. x 0.01 in., coated with Apiezon L and HI-EFF-4BP were used. Other conditions are the same as Figure 3.

A. Total fatty acids from Desulfovibrio Vulgaris, Hildenborough
B. Total fatty acids from Desulfovibrio Desulfuricans, Berre Sol
C. Total fatty acids from Desulforibro Desulfuricans, Essex 6
D. Total fatty acids from Yeast


A. DESULFOVIBRIO
VULGARIS,
HILDENBOROUGH
APIEZON L COLUMN
n-C16
i-C17
i-C15
n-C18
Δ11-C18
Δ9-C18
a-C17
n-C19
Δ9-C16
Δ7-C16
a-C15
n-C14
B. DESULFOVIBRIO
DESULFURICANS
BERRE SOL
HI-EFF-4BP
COLUMN
n-C17
n-C16
i-C15
Δ11-C18
n-C18
Δ9-C16
i-C17
a-C15
n-C12
Δ9-18
n-C19
a-C17
a-C13
i-C13
C. DESULFOVIBRIO
DESULFURICANS
ESSEX 6
APIEZON L COLUMN
n-C16
Δ9-C18
Δ9-C16
i-C15
n-C20
n-C18
i-C17
Δ7-C16
a-C15
C21
n-C19
Δ11-C18
n-C17
n-C15
n-C14
D. YEAST
HI-EFF-4BP COLUMN
Δ9-C16
n-C16
Δ9-C18
n-C18

XBL 703-5062

7. Summary

The morphology and physiology of living organisms are largely determined by genetic factors which presumably act through enzyme systems controlling chemical syntheses. Morphology, on which classification is normally based, is likely to depend on genetic constitution in a more complex way than that aspect of physiology which is concerned with the biosynthesis of relatively simple secondary constituents such as hydrocarbons and fatty acids. Each stage of such a synthesis requires an enzyme system which is genetically controlled and which can be modified or lost as the result of genetic mutation. Examination of the results of such mutations in terms of alterations of the structures of secondary products could therefore give information on evolutionary sequences, and to this extent it could be very useful aid to chemical taxonomy. The distribution of hydrocarbons have been discussed in Chapter 6. The taxonomy of fatty acids discussed previously in this chapter are generalized in Table IV.

The chemical taxonomic features are summarized as follows:

(a) Palmitic acid is generally present as a major component in all living organisms and it is found to be the most abundant saturated acid in all living organisms except several bacteria.

(b) Saturated and unsaturated C_{16} and C_{18} usually accounts for more than 90% of the fatty acids in algae and higher plants and animals, but less abundant in others.

(c) The odd numbered acids are only occasionally present in red and brown algae and bacteria but are absent in other living organisms.

(d) Branched acids other than iso-and anteiso-acids, are present in anaerobes, however, they have not been found in any other organisms.

(e) Iso- and anteiso-acids have only been detected in some of the fungus and anaerobic non-photosynthetic bacteria, they have not been seen in the others.

(f) Cyclopropyl acids are isolated in some of the photosynthetic and non-photosynthetic bacteria and absent in any other living organisms so far examined.

(g) Monounsaturated C_{16} and C_{18} are usually found in all living organisms. Δ-C_{18} is present to be the most abundant component in acid mixture in many occasions. In algae and higher living organisms mono-unsaturated C_{18} is present as Δ^{9}-C_{18} acid with or without the presence of a small amount of Δ^{11}-C_{18} acid, however, photosynthetic bacteria produce Δ^{11}-isomers.

(h) Polyunsaturated acids are the predominant constituents in green algae and higher plants, which have been detected in small amounts in blue-green algae. These acids are ranging from absent to trace in photosynthetic and non-photosynthetic bacteria.

Table IV

The Chemical Taxonomic Relationship of Fatty Acids Present in Living Organism

Straight Chain Saturated Acids

	C_{12}	C_{14}	C_{16}	C_{18}	$>C_{18}$	odd carbon number acid
Higher plants	generally absent	0-5%	major	present	occ. present	absent
Higher animals	generally absent	0-5%	major	present	absent	absent
Red and brown algae	generally absent	2-10%	26-40%	1-2%	4-6%	occ. present
Green algae	generally absent	occ. present	14-28%	0-4%	absent	absent
Blue-green algae	occ. present	generally absent	11-50%	0.3-5%	occ. present	absent
Fungal mycelia	generally absent	0.1-3%	12-34%	1-16%	0.7-5%	absent
Photosynthetic bacteria	0-3%	0-47%	3-36%	0.3-6%	occ. present	occ. present
Aerobic non-photosynthetic bacteria	present	present	major	present	absent	absent
Anaerobic non-photosynthetic bacteria	0-1%	0.2-85%	generally present as major peak 0.6-32%	0.2-10%	occ. present	occ. present

Table IV

The Chemical Taxonomic Relationship of Fatty Acids Present in Living Organism

Saturated Branch and Cyclic Acids

	iso-and anteiso-acids	branched other than iso-,ante-iso-acids	cyclopro-pyl acids	conjugated acid	hydroxy acid	epoxy acid
Higher plants	absent	absent	absent	occ. present	occ. present	occ. present
Higher animals	absent	absent	absent	absent	absent	absent
Red and brown algae	absent	absent	absent	absent	absent	absent
Green algae	absent	absent	absent	absent	absent	absent
Blue-green algae	absent	absent	absent	absent	absent	absent
Fungal mycelia	occ. present	absent	absent	absent	occ. present	occ. present
Photosynethetic bacteria	absent	absent	0-22%	absent	absent	absent
Aerobic non-photosynthetic bacteria	absent	absent	occ. present	absent	absent	absent
Anaerobic non-photosynthetic bacteria	generally present C_{13}-C_{17}	occ. present	occ. present	absent	absent	absent

Table IV

The Chemical Taxonomic Relationship of Fatty Acids Present in Living Organism

Unsaturated Acids

	Δ-C_{16}	Δ-C_{18}	polyunsaturated C_{16}	polyunsaturated C_{18}	polyunsaturated $>C_{18}$
Higher plants	1-5%	1-10%	0.1-5%	44-85%	0-25%
Higher animals	present	major	0-3%	1-5%	absent
Red and brown algae	present	present	0-1%	5-22%	15-40%
Green algae	present in small amt. Δ^7-C_{16}+Δ^9-C_{16}	2-54% major Δ^9-C_{18} some Δ^{11}-C_{18}	10-25% major 3Δ-C_{16}	30-55% major 3Δ-C_{18}	occ. present
Blue-green algae	3-61% present as Δ^9-C_{16}	2-31% major Δ^9-C_{18} some Δ^{11}-C_{18}	only 2Δ-C_{16} occ. present in small amount	generally present 2Δ-C_{18} 0.1-22% 3Δ-C_{18} 0-19%	absent
Fungal mycelia	1-12%	5-58%	1%	9-75% major 2Δ-C_{18} minor 3Δ-C_{18}	occ. present
Photosynthetic bacteria	1-51% present as Δ^9-C_{16}	0-92% dominant in most cases, present as Δ^{11}-C_{18}	generally absent	absent	absent

Table IV

The Chemical Taxonomic Relationship of Fatty Acids Present in Living Organism

Unsaturated Acids

	Δ-C_{16}	Δ-C_{18}	polyunsaturated C_{16}	polyunsaturated C_{18}	polyunsaturated $>C_{18}$
Aerobic non-photosynthetic bacteria	present as Δ^{9}-C_{16}	present as Δ^{11}-C_{18}	absent	traces	absent
Anaerobic non-photosynthetic bacteria	0.6-35% major Δ^{9}-C_{16} minor Δ^{11}-C_{16}	generally present Δ^{9}-C_{18} + Δ^{11}-C_{18}	absent	generally absent	absent

CHAPTER 7 REFERENCES

1. F. Lynen and S. Ochoa, Biochim. Biophys. Acta 12, 299 (1953).

2. D.E. Green, Biol. Rev. 29, 330 (1954).

3. J.F. Wintermans, Biochim. Biophys. Acta 44, 49 (1960).

4. H. Debuch, Z. Naturforschg. 16b, 561 (1961).

5. B.W. Nichols, Phytochemistry 4, 769 (1965).

6. E. Klenk, W. Knipprath, D. Eberhagen, and H.P. Koof, Physiol. Chem. 334, 44 (1963).

7. B.W. Nichols, Biochim. Biophys. Acta 106, 274 (1965).

8. J. Erwin, D. Hulanicka and K. Bloch, Comp. Biochem. and Physiol. 12, 191 (1964).

9. V.R. Williams and R. McMillan, Science 133, 459 (1961).

10. P.L. Parker, C. Van Baalen, and L. Maurer, Science 155, 707 (1967).

11. J.B. Wood, B.W. Nichols and A.T. James, Biochim. Biophys. Acta 106, 261 (1965).

12. G. Constantopoulos.

13. J. Nagai and K. Bloch, J. Bio. Chem. 237, 3702 (1965).

14. G. Scheuerbrandt and K. Bloch, J. Biol. Chem. 237, 2064 (1962).

15. K. Bloch, P. Baronowsky, H. Goldfine, W.J. Lennarz, R. Light, A.T. Norris, and G. Scheuerbrandt, Federation Proc. 20, 921 (1961).

16. K. Saito, J. Biochem. Tokyo, 41, 710 (1960).

17. T. Kaneda, J. Biol. Chem. 238, 1222 (1963).

18. K.J.I. Thorne and E. Kodicek, Biochim. Biophys. Acta 59, 306 (1962).

19. A. Ballio, S. Barcellona and L. Boniforti, Biochem. J. 94, 11C (1965).

20. S. Akashi and K. Saito, J. Biochem., Tokyo, 47, 222 (1960).

21. M.G. MacFarlane, Biochem. J. 82, 40P (1962).

22. M.J. Allison, M.P. Bryant, I. Katz and M. Keeney, J. Bact. 83, 1084 (1962).

23. G.H. Wegner and E.M. Foster, J. Bact. 85, 53 (1963).

24. R. Kaneda, J. Biol. Chem. 238, 1229 (1963).

25. T.G. Tornabene and J. Oró, J. Bact. 94, 349 (1967).

26. J. Asselineau, "The Bacterial Lipids", Holden-Day, Inc., San Francisco, p. 146, 1966).

27. H.W. Milner, J. Biol. Chem. 237, 2064 (1962).

28. B.W. Nichols, Biochim. Biophys. Acta 106, 274 (1965).

29. T-Y Liu and K. Hofmann, Biochemistry 1, 189 (1962).

30. J.H. Law, H. Zalkin and T. Kaneshiro, Biochim. Biophys, Acta 70, 143 (1963).

31. K. Hofmann, "Fatty Acid Metabolism in Microorganism," John Wiley & Son, New York, 1963.

32. W.M. O'Leary, "Transmethylation and Methionine Biosynthesis" (Shapiro S.K., and Schlenk, F., eds.), Univ. of Chicago Press, 1965, p. 94.

33. J. Han, H.W.-S. Chan and M. Calvin, J. Amer. Chem. Soc. 91, 5156 (1969).

34. F. Aylward and P.D.S. Wood, Nature 177, 146 (1955).

35. J. Cason and W.T. Miller, J. Biol. Chem. 238, 883 (1963).

36. T. Kaneshiro and A.G. Marr, Biochim. Biophys. Acta 70, 271 (1963).

37. D.G. Bishop and J.L. Still, Biochem. Biophys. Res. Commun. 7, 337 (1962).

38. N. Polgar and W. Smith, J. Chem Soc., 4262 (1962).

39. W.M. Crombie, J. Exp. Bot. 9, 254 (1958).

40. H. Debuch, Z. Naturforsch. 9, 561 (1961).

41. J.K. Heyes and F.B. Shorland, Biochem. J. 49, 503 (1951).

42. G.O. Burr and M.M. Burr, J. Biol. Chem. 82, 345 (1929).

43. Ibid., 86, 587 (1930).

44. H.E. Simmons and R.D. Smith, J. Amer. Chem. Soc. 31, 4256 (1939).

45. E. LeGoff, J. Org. Chem. 29, 2048 (1964).

46. F.W. McLafferty, Anal. Chem. 31, 82 (1959).

47. J.H. Beynon, "Mass Spectrometry and Its Applications to Organic Chemistry," Elsevier Publ. Co., Amsterdam, 1960.

48. R. Ryhage and E. Stenhagen, 'Mass Spectrometry of Organic Ions' (McLafferty, F.W., ed.), Academic Press, New York, 1963, p. 399.

49. K. Biemann, "Mass Spectrometry, Organic Chemical Applications," McGraw-Hill, 1962.

50. R. Ryhage and E. Stenhagen, Arkiv. Kemi, 15, 332 (1960).

51. P.L. Parker, Van Baalen C. and L. Maurer, Science 155, 707 (1967).

52. R.W. Holton, H.H. Blecker and M. Onone, Phytochemistry 3, 595 (1964).

53. B.W. Nichols, R.V. Harris and A.T. James, Biochem. Biophys.Res. Commun. 20, 256 (1965).

54. J.F. Slowey, L.M. Jeffrey and D.W. Hood, Geochim. Cosmochim. Acta 26, 607 (1962).

55. B.J.B. Wood, B.W. Nichols and A.T. James, Biochim. Biophys. Acta 106, 261 (1965).

56. K. Bloch, P. Baronowsky, H. Goldfine, W.T. Lennarz, R. Light, A.T. Norris, and G. Scheuerbrandt, Federation Proc. 20, 921 (1961).

57. T.G. Tornabene, E. Gelpi and J. Oró, J. Bacteriol. 94, 333 (1967).

58. T. Kaneda, J. Bacteriol. 93, 894 (1967).

59. T. Kaneda, J. Biol. Chem. 238, 1222 (1963).

60. K. Saito, J. Biochem., Tokyo, 47, 710 (1960).

61. M.D. Yudkin, Biochem. J. 82, 40 (1962).

CHAPTER 8 APPLICATION OF MASS SPECTROMETRY TO ORGANIC GEOCHEMISTRY

1. Introduction

The necessity of being able to unambiguously characterize the structures of organic compounds, isolated in microgram quantities from Precambrian sediments[1-3] and meteorites,[4-6] has been of utmost importance in the search for molecular fossils at the earliest periods of geologic time. Two techniques in particular, mass spectrometry and gas chromatography, (and more recently combined gas chromatography and mass spectrometry) have played a dominant role in the characterization of these compounds, and recent studies with Precambrian sediments[7] and with meteorites[8,9] have repeatedly utilized both of these analytical techniques.

The fully saturated isoprenoid hydrocarbons have received special consideration because it has been generally felt that the architecture of these molecules represented an unequivocal marker of biological origin.[10,11] Other schools of thought have challenged this fundamental premise and have sought to characterize these compounds in mixtures of known abiogenic origin. In this connection a recent report by Anders and his co-workers[12] seemed to us of particular significance, on account of his finding a series of isoprenoid structures, ranging in carbon number from C_9 to C_{14}, in a deuterium-carbon monoxide Fischer-Tropsch reaction. Another report by Schenck, Engelhardt and Göring[13] established the presence of a unique 2,6-dimethyl series in an Italian bituminous shale, also ranging in carbon number from C_9 to C_{14} and of presumed biological origin. This latter series was identical with the fully deuterated series characterized by Anders et al., except for the structural

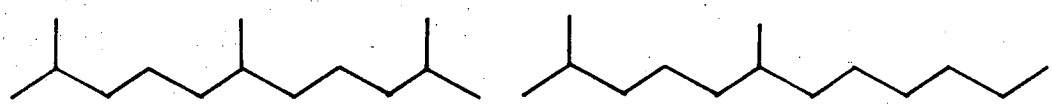
designation of the C_{14} compound. Our interest was drawn towards both of these reports, not only because they constituted one of the few examples where the lower molecular-weight isoprenoid hydrocarbons had been identified in either biogenic or abiogenic samples, but also because their published mass spectra showed a distinct similarity to the mass spectrometric fragmentation patterns of commercially available standards, which were structurally isomeric but non-isoprenoid in form. It therefore became imperative to study the fragmentation patterns of these isomers in greater detail and to establish criteria which might distinguish between them.

In certain cases ambiguities may arise in the structural interpretation of the saturated aliphatic hydrocarbons isolated from geochemical sources, and publications, both from this laboratory[7,11] and other laboratories[4,9] have emphasized the dangers inherent in designating a specific structure to a given compound on the basis of mass spectrometry alone. The availability of synthetic standards has generally resolved many of these uncertainties. This approach has proved extremely fruitful but at times it has turned out to be a time-absorbing operation when the hydrocarbon standards cannot be readily obtained. For this reason also, as well as for the reason stated above, we have explored some apparently anomalous features in the mass spectrometric fragmentation patterns with the view of exploiting them for diagnostic proposes.

2. Methyl-alkanes

The mass spectra of the C_9 isomeric hydrocarbons, 2,6-dimethylheptane and 2-methyloctane, the C_{10} isomeric hydrocarbons, 2,6-dimethyloctane and 3-methylnonane, and the C_{11} hydrocarbons, 4-methyldecane, isomeric with the

2,6-dimethylnonane structure reported by both Anders et al.[12] and Göhring et al.[13] are shown in Figures 1, 2 and 3 respectively. The similarities in the mass spectra of these isomeric hydrocarbons are quite evident, both C_9 isomers showing intense ions at m/e 113 and subsequently ions of increasing intensity from m/e 99 to m/e 43, for each 14 mass unit decrease. An analogous pattern is observed for the C_{10} isomeric hydrocarbons, where again m/e 113 peak is a dominant fragmentation mode, and likewise for the C_{11} hydrocarbons, 4-methyldecane, whose mass spectrum shows resemblance to the published spectra of 2,6-dimethylnonane.[13] This evidence indicates that the moving of a methyl group from the 2-position to the end of the hydrocarbon chain causes very minor differences in the mass spectra of the two compounds. An identical effect had been previously observed by us[14] with the C_{19} isoprenoid isomers, pristane and 2,6,10-trimethylhexadecane. This kind of relationship allows one to predict that the mass spectra of 2,6,10-trimethylundecane (I) and 2,6-dimethyldodecane (II) should be very similar:



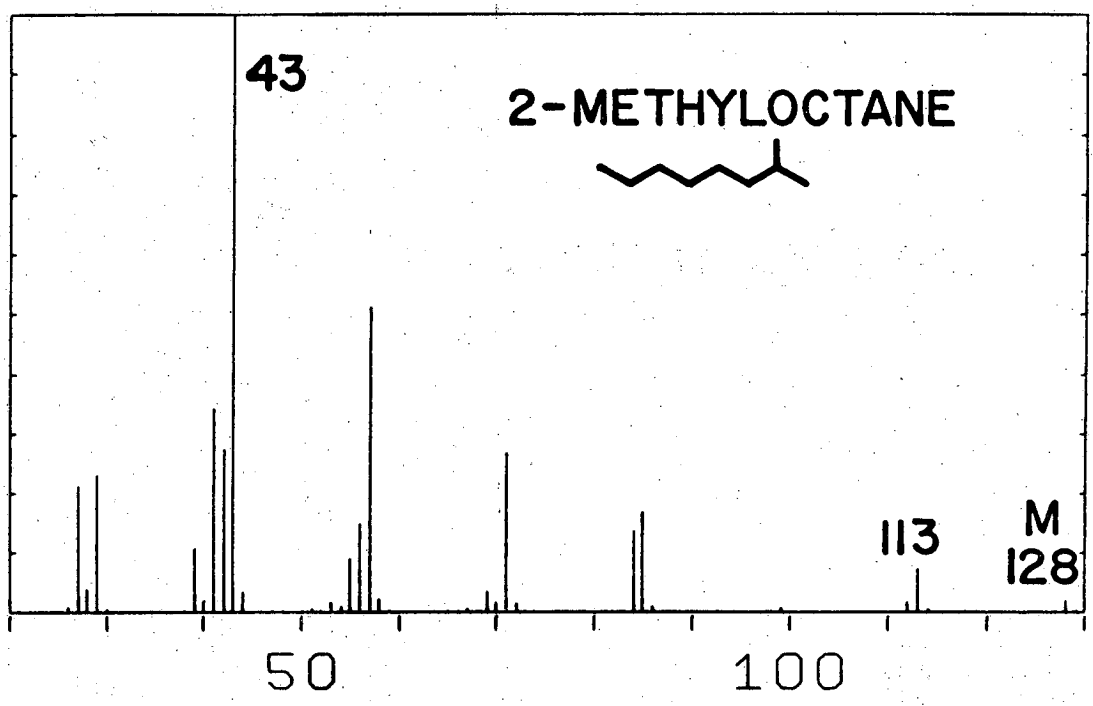
I II

The only mass spectral comparison that is available in the literature** would seem, in its gross features, to justify such a prediction.

The structural identity of each of these isomers is easily determined using infrared spectroscopy, NMR spectroscopy or gas-liquid chromatographic

**(Fully deuterated (I) is reported by Anders et al.[12], (II) has been described by Göhring et al.[13].

Figure 1. Mass spectra of 2-methyloctane and 2,6-dimethylheptane. Spectra were taken using an A.E.I. MS-12 mass spectrometer. The spectra were determined at ionizing voltage 70 ev, and ionizing current 50μA. The volatile compounds were put in with a glass inlet system which was operated at room temperature, with the probe pre-cooled in liquid nitrogen. The temperature of ion source was 200°C. Each compound was scanned in 30 seconds. The spectra were recorded by an oscillograph recorder.



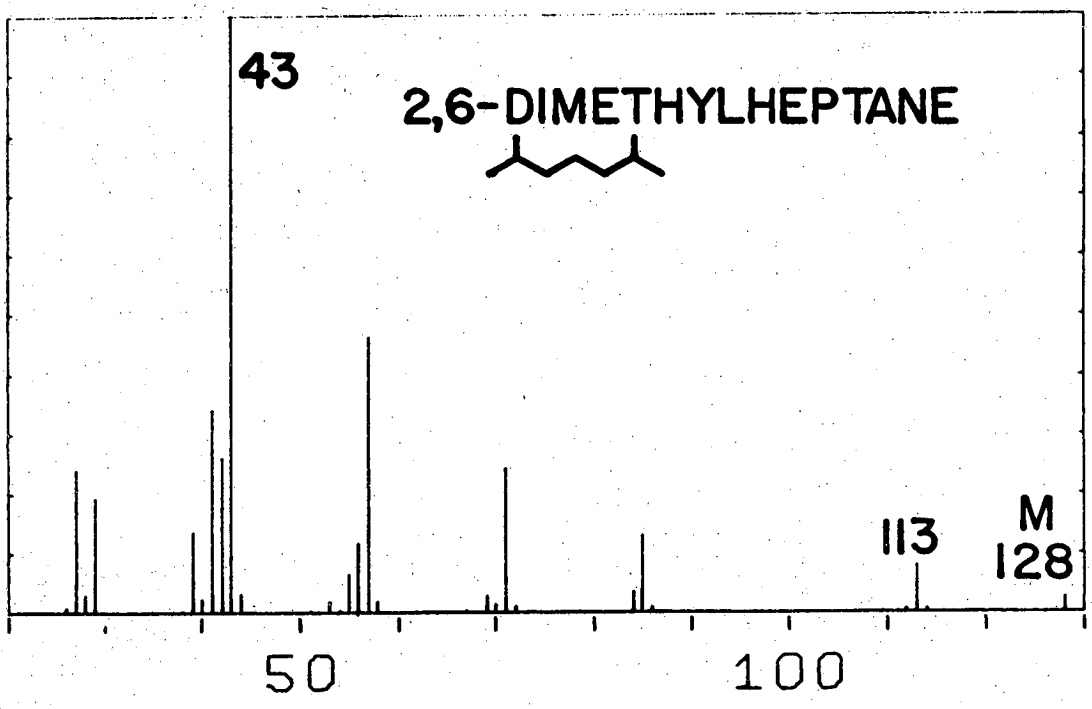


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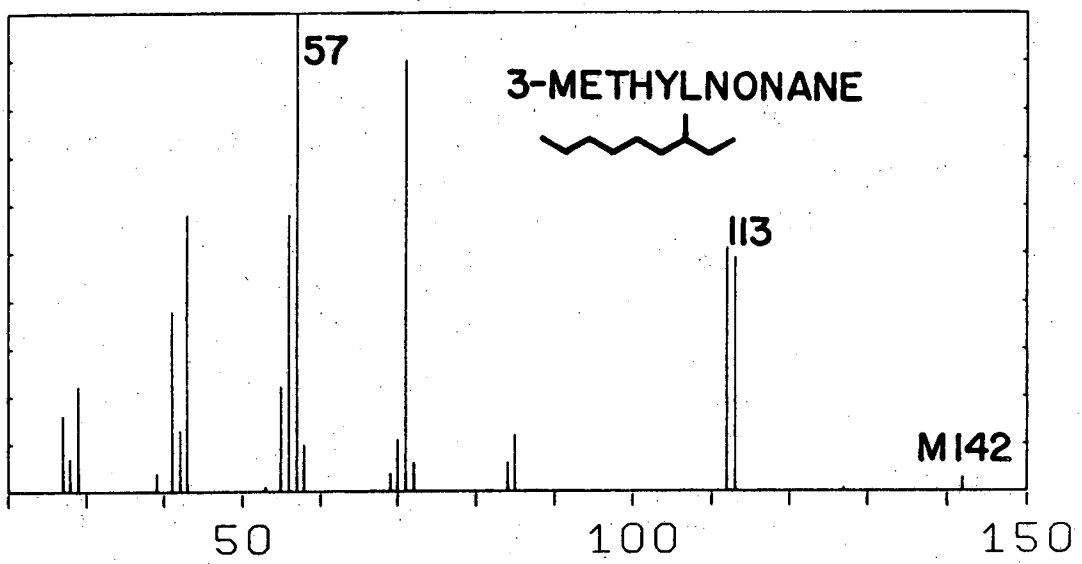
Figure 2. Mass spectra of 3-methylnonane and 2,6-dimethyloctane. All the conditions are the same as Figure 1.



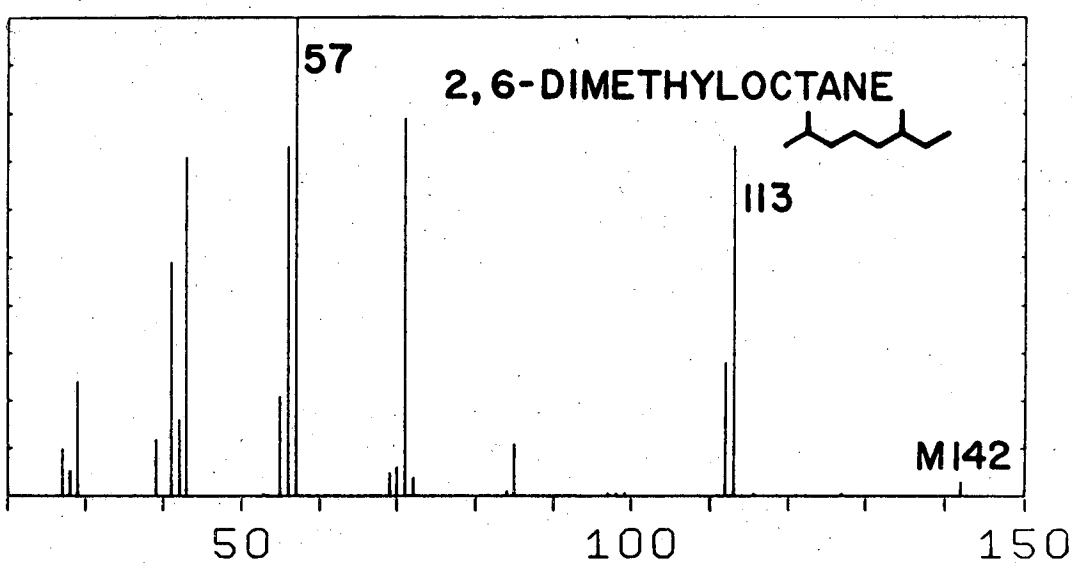


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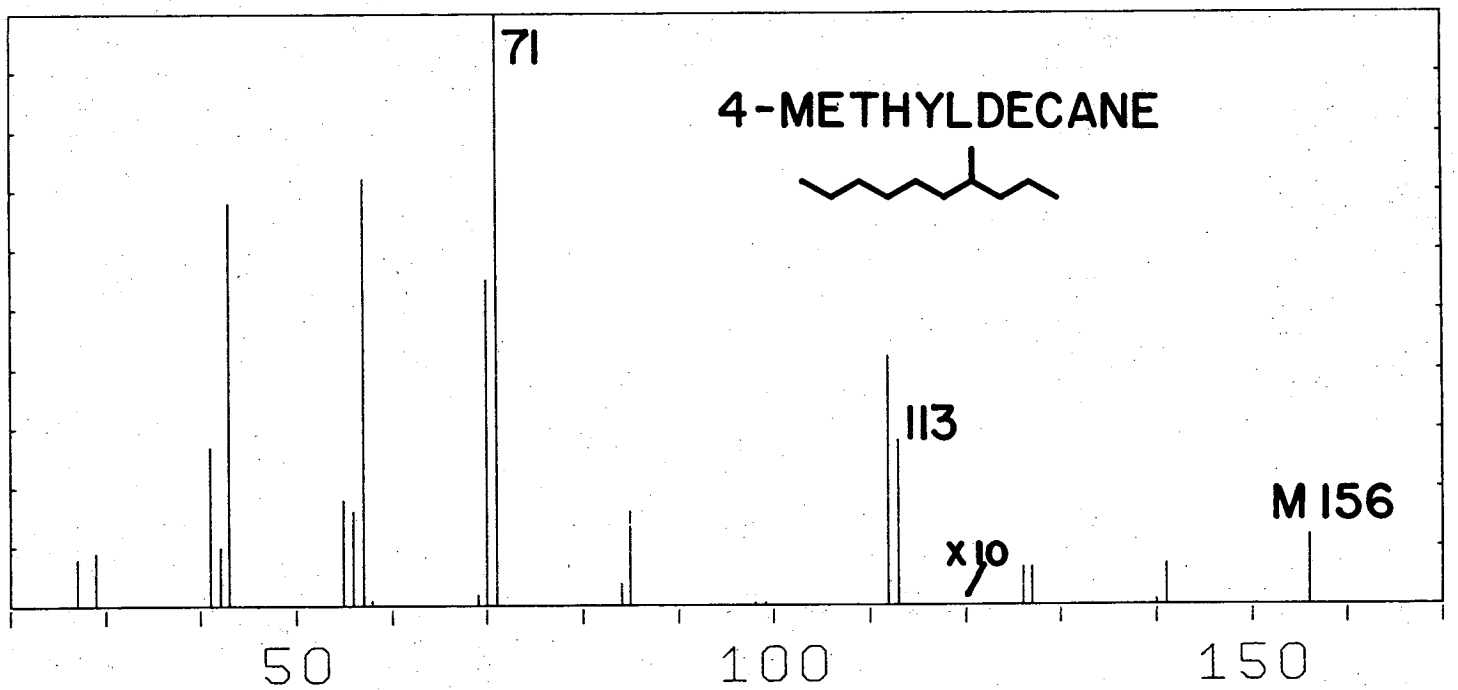
Figure 3. Mass spectrum of 4-methyldecane. All the conditions are the same as Figure 1.



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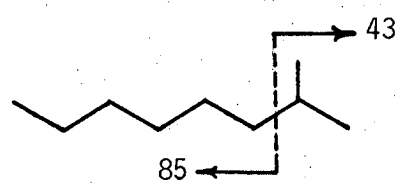
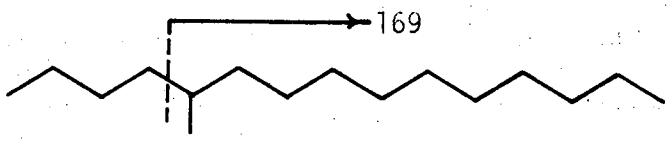
retention times. This approach, however, is dependent on each constituent being present in sizeable amounts for the spectroscopic procedures, and on the availability of hydrocarbon standards for GLC retention times. Since neither of these prerequisites are fulfilled in many organic geochemical studies we determined to re-examine the mass spectrometric fragmentation patterns of these isomers in the hope of distinguishing between them.

The difference between the mass spectra of 2-methyloctane and 2,6-dimethylheptane lies in the relatively high intensity of the m/e 84 peak, for 2-methyloctane, giving the appearance of a pair of peaks at m/e 84 and m/e 85. This pair is not as marked in the mass spectrum of 2,6-dimethylheptane. The m/e 85 peak presumably corresponds to the fragmentation shown, with the primary fragment retaining the charge.



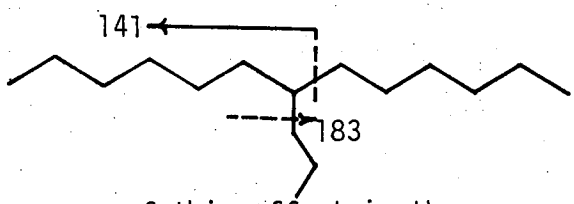
An analogous effect appears to be present in the mass spectra of 3-methylnonane and 4-methyldecane where a pair of peaks occur at m/e 112 and m/e 113. For these last two compounds the C_8 fragment intensity is high because the charge now resides on a secondary carbon atom. In neither the mass spectrum of 2,6-dimethyloctane nor the 2,6-dimethylnonane mass spectrum of Göhring *et al.*[13] does the m/e 112 peak exhibit a comparable intensity. The occurrence of such pairs in the fragmentation patterns of branched hydrocarbons

had previously been observed by Biemann,[15] who reports that some fragments have "a tendency to lose a hydrogen atom giving rise to a doublet of peaks." In the mass spectrum of 5-methylpentadecane(III) the preferred fragment at m/e 169 will lose a hydrogen giving rise to a peak at m/e 168 whose intensity is greater than that of the corresponding odd peak.



(III)

This effect is also observed with 7-n-propyltridecane (IV) (A.P.I. #591) where the preferred fragmentations at m/e 183 and m/e 141 give rise to peaks at m/e 182 and m/e 140 of equal, if not greater, intensity.



(IV)

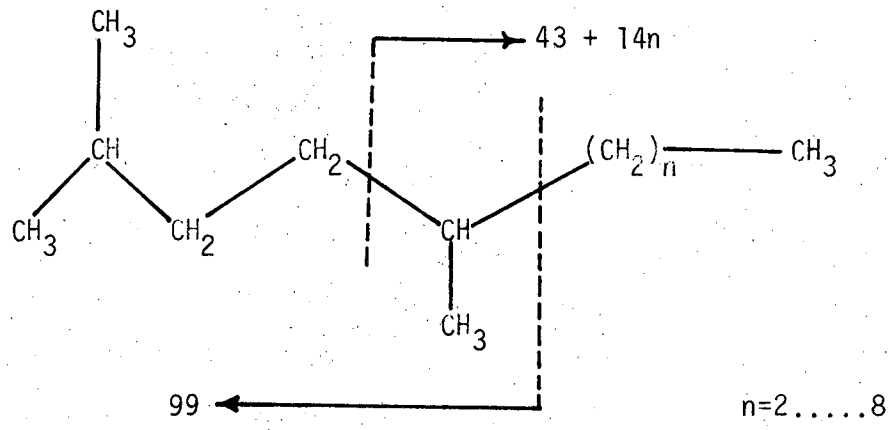
The occurrence of this effect in the mass spectra of several acyclic hydrocarbons containing a single alkyl branch prompted us to search for the same effect in the dialkyl hydrocarbon series. We had already noticed its absence in the C_9 and C_{10} 2,6-dimethyl hydrocarbons, but a study of the mass spectra of a 2,5-dimethyl hydrocarbon series (A.P.I. #1942-1948), ranging from C_{10} to C_{16} in carbon number, provided further insights into the occurrence of this pair of peaks. Table I illustrates the increasing intensity of the even peak relative to the odd peak for the fragmentation, (43 + 14n), shown below.

TABLE I

2,5-Dimethyl Alkane Mass Spectra

A.P.I. #	Compound	m/e (43 + 14n)				n	m/e 98	m/e 99
		even	intensity	odd(m/e)	intensity			
1942	2,5-dimethloctane	70	30.1	71	35.6	2	7.93	14.1
1943	2,5-dimethylnonane	84	16.4	85	28.4	3	6.47	11.3
1944	2,5-dimethyldecane	98	13.0	99	10.6	4	13.0	10.6
1945	2,5-dimethylundecane	112	9.37	113	5.26	5	5.14	8.72
1946	2,5-dimethyldodecane	126	9.18	127	5.98	6	7.67	10.2
1947	2,5-dimethyltridecane	140	8.04	141	6.17	7	7.12	13.6
1948	2,5-dimethyltetradecane	154	6.93	155	5.97	8	7.16	15.3

Base Peak: 57 = 100



We have also observed an identical effect in the mass spectra of the 2,6-dimethyl series published by Göhring *et al.*[13]

Two generalizations may be drawn from this comparative study. *In the first place* the occurrence of a pair of peaks appears to be dominant in the fragmentations giving rise to ions which contain no other branches. *In the second place* the even peak will generally be more intense than the odd peak when the fragment ion contains seven or more carbons in the chain. Both these effects can be seen in the mass spectra of the 2,5-dimethyl series. An alternative fragmentation to the (43 + 14n) peak is the m/e 99 peak, shown above, containing one methyl branch. This fragmentation mode does not exhibit a marked doublet of peaks (Table I) in which the ratio of the even to odd intensities increases with increasing n.

To substantiate these generalizations we proceeded further to examine other branched hydrocarbons in the mass spectral tables of the American Petroleum Institute. A thorough survey of the mass spectra of these branched hydrocarbons fully vindicated our preliminary conclusions. The ions resulting from fragmentations at the branch positions in the chain are shown in Table II.

TABLE II

Branched Alkane Mass Spectra

A.P.I. #	Compound	Even (m/e)	Intensity	Odd (m/e)	Intensity	Base Peak
2018	5-n-butylnonane	126	42.8	127	18.8	43
	5,8-diethyldodecane	98	31.27	99	21.45	71
2019	4,9-di-n-propyldodecane	98	50.4	99	24.9	71
704	7-methyltridecane	112	29.4	113	14.3	57
491	7-n-propyltridecane	140	23.1	141	12.9	57
		182	9.18	183	11.7	
592	7-n-hexyltridecane	182	29.1	183	23.9	57
1469	8-n-hexylpentadecane	196	26.20	197	23.31	71
		210	12.31	211	12.62	
1470	5-n-butylhexadecane	126	21.91	127	12.68	71
		224	15.94	225	20.72	
578	6,11-di-n-pentyl-hexadecane	154	24.0	155	11.1	57
1320	9-n-hexylheptadecane	210	18.49	211	15.89	71
		238	11.80	239	14.46	
983	9-n-octylheptadecane	238	19.30	239	20.91	71
593	4-n-propylheptadecane	98	19.5	99	9.84	57
		238	9.40	239	13.8	
1032	5,14-di-n-butyl-octadecane	126	23.3	127	13.8	71
1257	7-n-hexyleicosane	182	12.56	183	11.01	71
		280	8.29	281	11.31	
1472	3-methyleicosane	154	16.15	155	10.75	71
		168	13.76	169	10.56	
1474	9-n-octyleicosane	238	12.87	239	13.51	71
864	11-n-pentylheneicosane	224	22.7	225	19.9	43
		294	7.86	295	12.3	
579	11-n-decylheneicosane	294	18.7	295	24.2	57
1256	11-(3-pentyl)-heneicosane	294	4.30	295	4.57	71

TABLE II (CONT.)

A.P.I.#	Compound	Even (m/e)	Intensity	Odd (m/e)	Intensity	Base Peak
861	5-n-butyldocosane	126 308	24.7 11.2	127 309	10.7 18.2	43
706	7-n-butyldocosane	154 280	14.1 4.30	155 281	8.13 5.42	57
862	9-n-buthydocosane	182 252 308	15.6 8.83 7.11	183 253 309	10.9 9.06 13.0	43
863	11-n-butyldocosane	210 224 208	12.9 11.6 7.29	211 226 309	10.5 10.0 13.6	43
865	7-n-hexyldocosane	182 308	19.1 15.8	183 309	12.9 12.5	43
866	9-n-octyldocosane	238 308	11.1 13.3	239 309	10.4 17.1	43
867	11-n-decyldocosane	294 308	10.1 17.7	295 309	11.6 21.3	43
1259	11-n-decyltetracosane	294 336	6.87 8.92	295 337	8.17 12.65	71
1475	9-n-octyltetracosane	238 336	15.43 14.57	239 337	15.38 22.36	71
1355	13-n-undecylpentacosane	336	16.79	337	23.17	71
1476	13-n-dodecylhexacosane	350 364	7.67 13.15	351 365	10.72 19.25	71
1322	9-n-octylhexacosane	238 364	13.28 8.89	239 365	12.91 14.63	71
1357	11,20-di-n-decyltricontane	294	35.70	295	26.34	85

In every case a pair of peaks occurs, and in most of them the even peak is more intense than the odd peak. It is emphasized that such a plot is compiled from a sizeable number of branched hydrocarbons of widely differing structures, where the only restriction has been the non-branched character of the fragment ion. The even peak is seen to be particularly dominant when the fragment ion contains 7,8 or 9 carbon atoms. Thus the even peaks of the branched hydrocarbons 4-n-propylheptadecane (#593), 7-methyltridecane (#704) and 5-butylnonane (#2018) are more than twice the intensity of the corresponding odd peaks. These three hydrocarbons have major fragment ions at m/e 99 (C_7), m/e 113 (C_8) and m/e 127 (C_9) respectively. When the fragment ion contains more than nine carbon atoms the ratio of the intensities appears to decrease in magnitude, and when the fragment ion contains 18 or more carbon atoms the odd peak will be generally more intense than the even peak. (See for example 9-n-butyldocosane (#862), 7-n-hexyleicosane (#1257) and 11-n-decyldocosane (#867), though the C_{22} fragment in 7-n-hexyldocosane (#865) is an exception). Thus, even peaks are of the same order of intensity as the odd ions containing from 7 to 17 carbon atoms (Table II).

The generalizations that we have made do appear to have some additional restrictions. The occurrence of a pair of peaks is never observed in a dialkyl substituent when both substituents are located on the same carbon atom. Thus 5-ethyl, 5-propylundecane (#1543) and 5-methyl 5-ethylundecane (#1544) give rise to ions that show <u>no</u> tendency to lose a hydrogen atom. Further examples of this type of compound serve to confirm this observation (#1544 to #1553). The <u>iso</u>-alkane series (2-methyl alkanes) shows anamalous behavior. In general <u>iso</u>-alkanes have two characteristic fragmentations,

an (M-15) ion and an (M-43) ion; the latter ion does display a tendency to lose a hydrogen atom for the lower molecular weight members of the series. This is illustrated in Table III. The limited examples that are available for the anteiso-series (3-methyl alkanes) suggests a similar anomalous behavior here also (see for example #1472). In contrast to these limitations the vicinal dimethyl hydrocarbons seem to show an enhanced tendency to lose a hydrogen. Thus 2,3-dimethylheptane (#337) and 3,4-dimethylheptane (#342) have very intense m/e 84 and m/e 70 ions, relative to m/e 85 and m/e 71. to m/e 85 and m/e 71.

#337, 2,3-dimethylheptane	m/e 84 = 28.1; m/e 85 = 24.2
#342, 3,4-dimethylheptane	m/e 70 = 46.1; m/e 71 = 29.7
#480, 2,3-dimethyloctane	m/e 98 = 28.4; m/e 99 = 7.72

The applicability of these observations to organic geochemical studies is extensive. A closer look at the mass spectra of several isoprenoid hydrocarbons reveals the occurrence of the effect in this series also. Thus the mass spectrum of 2,6,10-trimethylhexadecane can be distinguished from that of pristane, 2,6,10-^{14}tetramethylpentadecane, on the basis of the relative intensities of the m/e 112 and the m/e 113 ions.[11] One would predict that m/e 112 peak should be more intense than the m/e 113 peak for the 2,6,10-trimethyl isomer, and this is in fact observed. A similar prediction would be made for the C_{18} isoprenoid hydrocarbon 2,6,10-trimethylpentadecane.

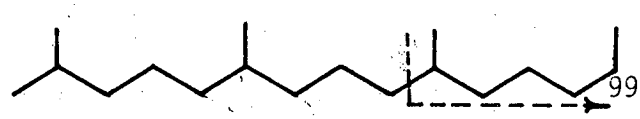


TABLE III

Iso-Alkane Mass Spectra

A.P.I.#	Compound	Even (m/e)	Intensity	Odd (m/e)	Intensity	Base Peak
15	2-methylhexane	56	20.1	57	26.0	43
40	2-methylheptane	70	17.1	71	12.8	43
245	2-methyloctane	84	13.5	85	16.7	43
479	2-methylnonane	98	12.2	99	8.86	43
840	2-methyldecane	112	9.72	113	9.67	43
982	2-methylpentadecane	182	4.67	183	13.7	43
983	2-methylheptadecane	210	3.80	211	12.7	43

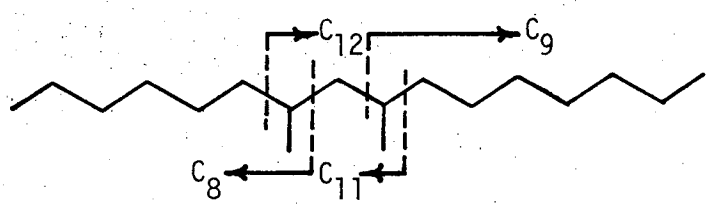
TABLE IV

Mass Spectra of C_{18} Isoprenoid Hydrocarbons

Ref.	Source	Even (m/e)	Intensity	Odd (m/e)	Intensity	Base Peak
21	Moonie Oil	98	140	99	130	113
22	Green River	98	140	99	100	113
2	Soudan	98	109	99	110	113
2	Antrim	98	117	99	123	113
23	East Texas	98	9.22	99	8.40	57
24	D'Arcy Oil	98	12	99	13	57
25	Standard	98	88	99	88.5	113

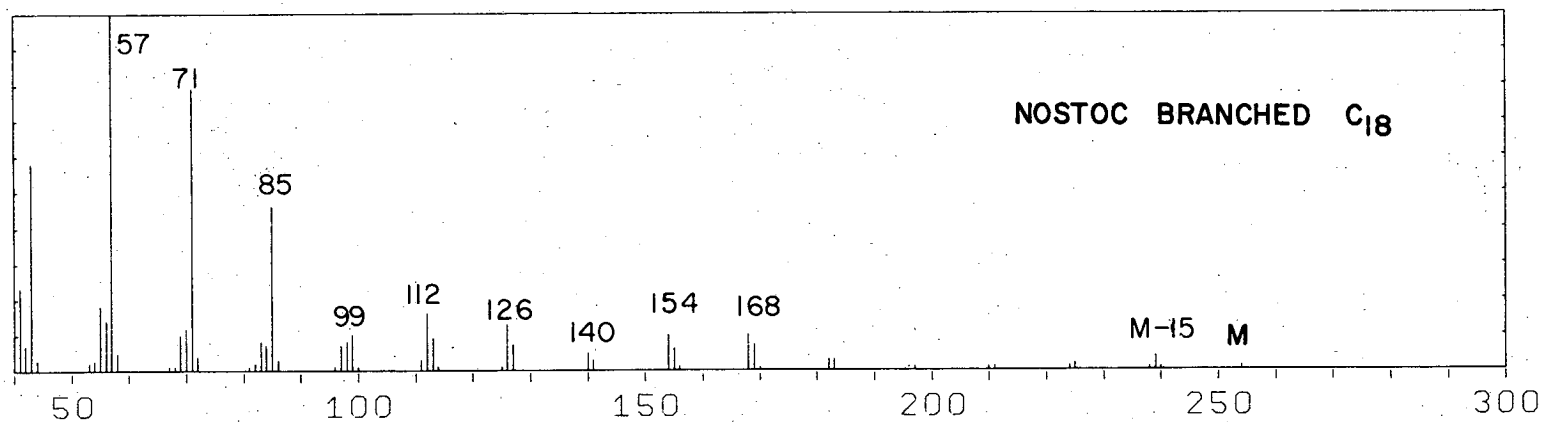
Here again one would anticipate that the m/e 98 ion should be an intense peak, as intense, if not more intense, than the m/e 99 ion; this prediction is borne out, as can be seen in Table IV. The C_{18} isoprenoid hydrocarbons were isolated from different geological samples. This is all the more surprising since all except one of the C_{18} isoprenoid hydrocarbons in Table IV are not 100% pure, being isolated from complex hydrocarbon mixtures. There is a danger in attributing too much significance to the lower molecular weight ions in the mass spectra of isoprenoid hydrocarbons since they may arise from a degeneracy of fragmentation modes or, alternatively, by further decomposition of larger hydrocarbon fragments (type A_2 c.f. Biemann.[18]

The value of these observations has perhaps been most strikingly demonstrated in assigning a specific structure to a C_{18} branched hydrocarbon isolated from the blue-green alga, Nostoc. The mass spectrum of this hydrocarbon is shown in Figure 4. It exhibits four major fragments, C_8, C_9, C_{11} and C_{12}, corresponding to fragment ions at m/e 112, 113, m/e 126, 127, m/e 154, 155 and m/e 168, 169. The even ion is always more intense than the odd ion. One structure consistent with such a mass spectrum would be 7,9-dimethylhexadecane, a structure we had tentatively proposed in a previous report.[19]



In the light of our previous discussion and from the generalizations that we subsequently derived one would anticipate the occurrence of a doublet

Figure 4: Mass spectrum of branched-C_{18} hydrocarbon from Nostoc.



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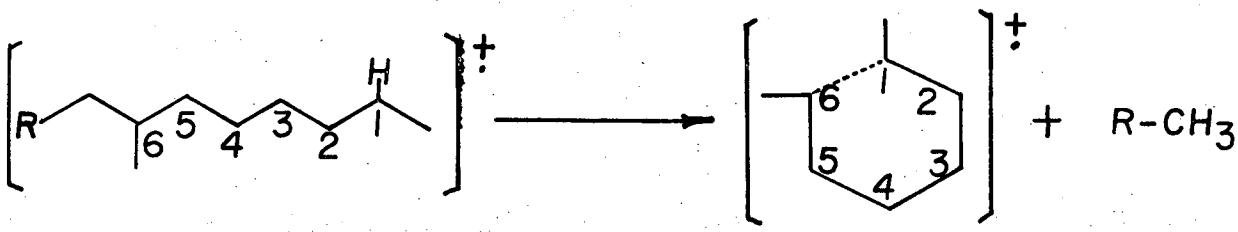
of peaks for the C_8 and C_9 fragment ions, and this is, in fact, observed. It is somewhat surprising to find the same effect for the C_{11} and C_{12} fragment ions, since these ions contained a methyl branch, and in such structures the loss of a hydrogen atom to produce an even ion is generally not as dominant as the mass spectrum of Figure 4 would suggest. Synthesis of the pure diastereoisomers of 7,9-dimethylhexadecane followed by capillary coinjection techniques confirmed our suspicion that the C_{18} branched hydrocarbon isolated from the blue-green alga, Nostoc, did not have this structure. The only other feasible explanation was that the Nostoc C_{18} branched hydrocarbon consisted of an equal mixture of 7-methyl and 8-methylheptadecanes, which we could not separate by capillary gas-liquid chromatography. Such a mixture would be expected to give rise to doublets of peaks at C_8 and C_{12}, for the 7-methyl compounds, and C_9 and C_{11}, for the 8-methyl compound, in which the even ion is more intense than the odd ion in every case. Synthesis of these two methylheptadecanes fully vindicated our prediction.[14]

This chapter constitutes an empirical correlation that is of considerable diagnostic value in the structural characterization of branched hydrocarbons from their mass spectral fragmentation patterns. Little work has been reported which provides a deeper insight into the fragmentation mechanism of this hydrogen transfer,[20] which is to be expected from a consideration of the favourable energetics. A possible mechanism is shown below in which hydrogen transfer from a secondary position takes place to form a cis 1,2-dialkylcyclohexane ion and an alkane. The metastable peaks correspond to the loss of $R\text{-}CH_3$ groups and the formation of C_nH_{2n} ions is observed.



This situation might be expected to prevail when the charge carrying fragment has a sufficiently long unbranched chain to allow it--provided the alkane formed is not methane c.f. (M-15) ion in the iso-alkane series.

In addition, this mechanism lends itself to a specific prediction when suitable deuterated materials are available. We are at present investigating this aspect of the fragmentation mechanism.

3. 1-Alkene (double bond on the terminal carbon)

1-Heptadecene was isolated from *Chlorella pyrenoidosa*, and the structure was proved by the oxidative degradation reaction. When the mass spectrum of 1-heptadecene was examined carefully, a high intensity peak, M-28 could be observed which was higher than the proceeding fragment. Fig. 4 shows the mass spectrum of *Chlorella* 1-heptadecene. The M-28 peak corresponded to the loss of an ethylene molecule; it was not found in any of the mass spectra of saturated hydrocarbons. Little work had been done elsewhere.[26] A search through the olefinic spectra in the A.P.I. Tables shows (Table V) that this is indeed the case.

The first possibility is vinyl bond cleavage and a hydrogen atom is picked up by the vinyl group. Thus it forms an M-28 fragment and an ethylene molecule. Usually, an allylic cleavage of an olefinic hydrocarbon is thermodynamically

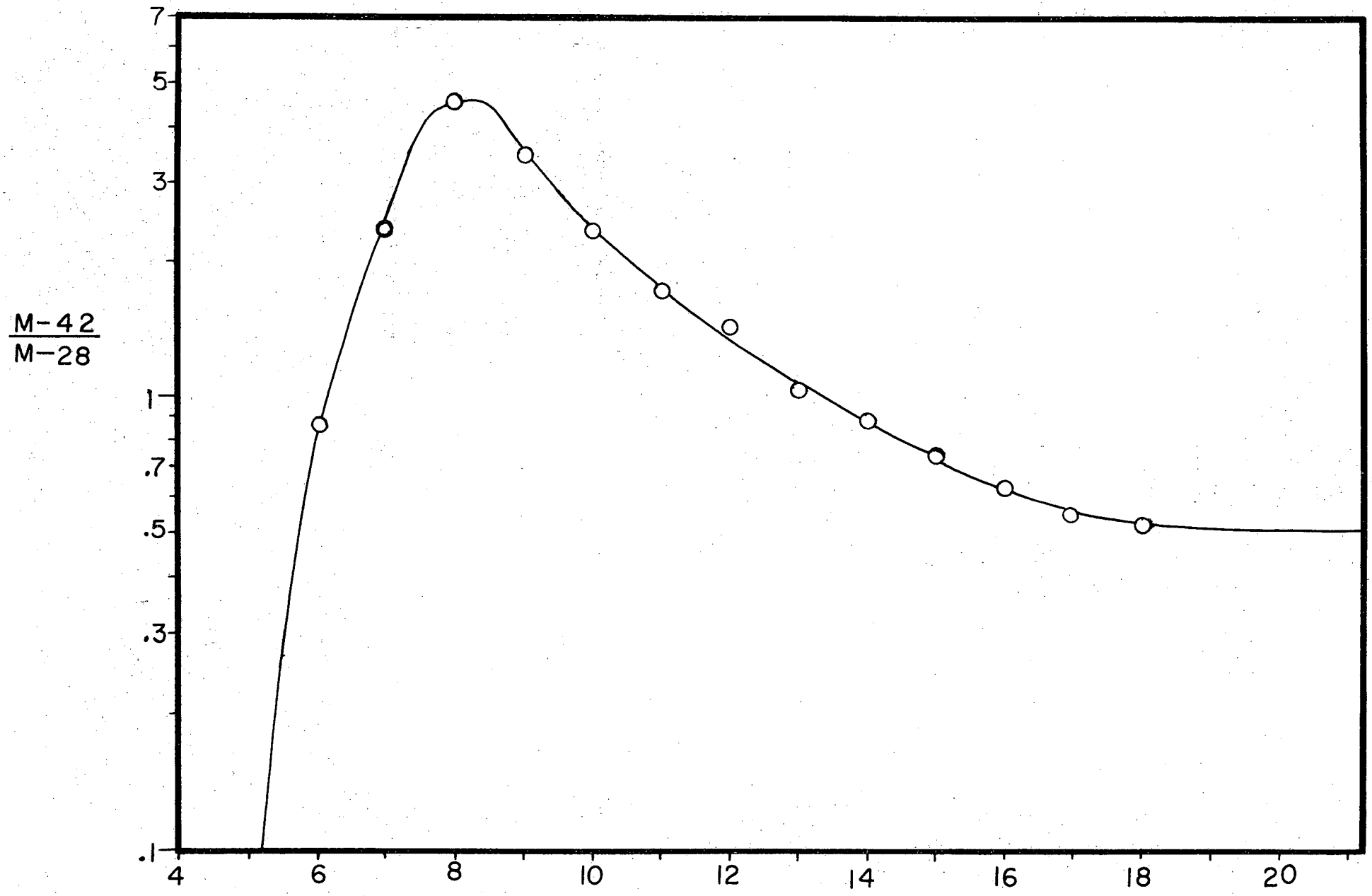


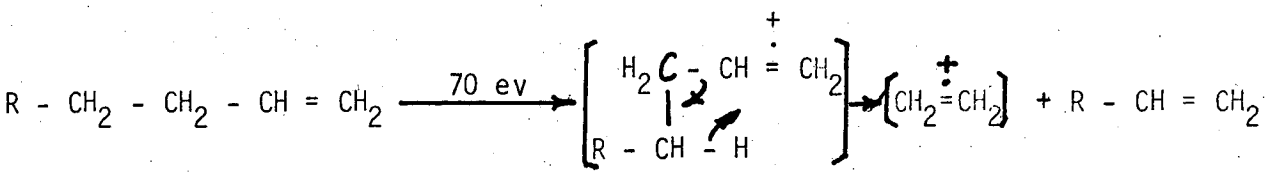
FIG. IV

TABLE V

MASS SPECTRA OF n-ALKENE-1

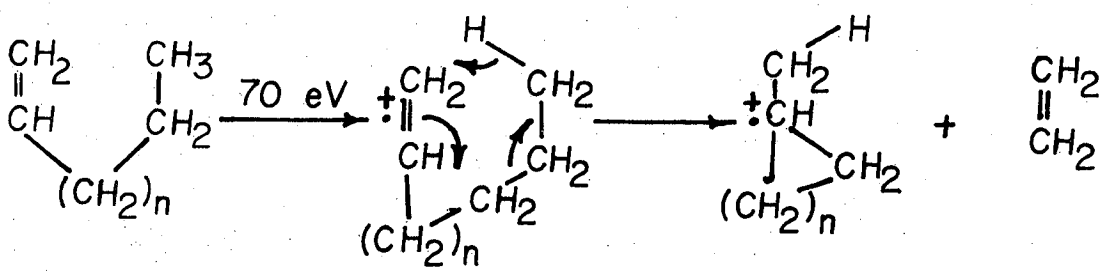
A.P.I.#	Compound	M	M-13	M-14	M-15	M-27	M-28	M-29	M-41	M-42	M-43	Base Peak m/e
67	1-Butene	46	0.12	3.47	100	10.7	22.6	19.5	.95	.26	.10	41
152	1-Pentene	30.9	.07	2.65	60.1	4.09	100	44.3	27.1	4.54	32.1	42
98	1-Hexene	28.2	.02	1.08	18.7	3.88	86.0	59.4	59.7	75.1	100	41
107	1-Heptene	16.4	.03	.25	3.54	1.92	36.8	26.7	26.4	87.3	59.8	41
128	1-Octene	10.8	--	.17	2.11	.78	13.4	20.5	8.69	61.2	30.5	43
240	1-Nonene	7.34	--	.04	.45	.40	5.10	12.6	3.45	20.8	17.3	43
241	1-Decene	6.42	--	.02	.18	.35	4.55	6.26	.81	10.9	.517	41
407	1-Undecene	4.0	--	--	.07	.22	2.65	2.05	.40	4.55	5.83	41
485	1-Dodecene	2.15	--	--	--	.17	1.50	.41	.21	2.10	1.78	41
1010	1-Tridecene	4.72	--	.04	.19	.51	4.03	.92	.57	4.22	2.27	41
1011	1-Tetradecene	4.04	--	--	.03	.47	3.35	.61	.40	2.91	1.26	41
1012	1-Pentadecene	3.53	--	0.02	.03	.42	2.95	.38	.36	2.18	.90	41
1013	1-Hexadecene	3.79	--	--	.02	.42	2.51	.30	.32	1.59	.59	41
20-M	1-Heptadecene	441	--	--	--	31	173	19	18	95	42	41
21-M	1-Octadecene	469	5	16	4	32	173	19	21	93	24	41

favored but the vinyl cleavage is the least expected. I would like to propose a possible vinyl bond cleavage route, a four center mechanism as follows:



After electron bombardment in the ion source, the charged particle is favored to stay in a P-orbital, the double bond position. In order to cleave the vinyl bond, the charge cannot migrate to the other part of the molecule. A charged ethylene molecule and a new olefinic hydrocarbon should be formed. However, that is not the case.

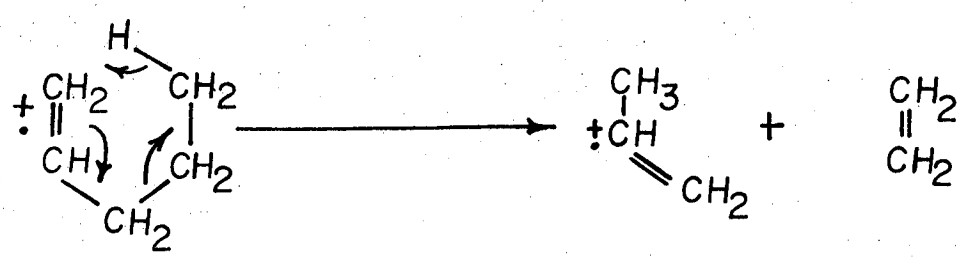
The other reasonable possibility is to form a charged cyclic fragment and split out an ethylene molecule. A six-center mechanism is proposed as follows:



This mechanism involves a head-to-tail six-member ring transition state and then splits out two carbons from the eail end. This mechanism is particularly reasonable not only because it has a six-member transition state, but also because the charge stays on the tertiary carbon atom of the fragment product.

Table V has been examined very carefully. An M-28 peak has a relatively high intensity for the low mass molecules, but its intensity drops down and then rises again when the molecular weight of the olefin is increasing. A curve, M-42 to M-28 ratio against the carbon number of the molecule is shown in Fig. IV. The curve increases so rapidly from C_5 to C_8 and it has a maxima at C_8 or C_9. The curve decreases slowly and has a tendency to keep constant at the higher molecular end. This curve suggests the head-to-tail ring intermediate could be involved because this curve coincides to the ΔH of ring formation. At medium range, C_8 to C_{10}, the ring is very strained making this mechanism less favored.

n-Pentene has a much higher intensity of M-28 fragment than expected, because it forms a different product as follows:



A charged propene and an ethylene should be produced. The charge rests on the P-orbital of propene, which is more stable than it is on the tertiary carbon of the cyclic fragment. A deuterated compound is suggested to prove this mechanism.

4. Summary

The general characteristics of mass spectrometric fragmentation patterns of aliphatic hydrocarbons have been reported elsewhere[26-]. The important features are summarized as follows.

a. Normal Alkane

i. It does show a small molecular ion.

ii. The total carbon number can be calculated as Intensity of Molecular Ion $(C_nH_{2n}+2)/1.08$ x Intensity of Molecular Ion + 1 (CnH_2n+3).

iii. The highest mass peak in each carbon number region is CnH_2n+1.

iv. Generally the carbon fragment groups range from $C_nH_{2n}-2$ to C_nH_2n+4. The mass difference between two adjacent maximas is 14.

v. The distribution curve has a maxima around C_4 or C_5 fragments, and then it rapidly decreases to the high mass region.

vi. The M-15 fragment is either absent or very small in the spectrum since the single cleavage of the terminal methyl group is not a favorable process.

b. Normal Alkene (mono olefin).

i. The fragmentation pattern is similar to that of the n-alkane, but its molecular ion has a higher intensity than the saturated one.

ii. If the double bond is on the perminal position and the molecule contains more than 13 carbons, there is an outstanding M-28 peak.

iii. The highest mass peak in each fragmentation group is $CnH_{2n}-1$ in the low mass region, but $C_nH_{2n}+1$ in the high mass region.

c. Branched Alkane.

i. The relative intensity of the molecular ion is lower.

ii. The low of the methyl peak, M-15 is usually higher than the molecular ion.

iii. Carbon-carbon bond breaking always takes place at a quarternary or tertiary carbon center; after splitting the charge tends to stay at tertiary or secondary carbon atoms.

iv. If there are more than two alkyl groups bonded with one carbon atom, the loss of the small group is more favored than the splitting out of a large alkyl group.

v. After bond cleavage adjacent to a methyl branch the charge normally stays on the secondary carbon. If the primary fragment is sufficiently large, the charge can sometimes remain on the primary carbon fragment.

vi. The fragmentation of a saturated aliphatic hydrocarbon would cause a hydrogen transfer giving rise to a C_nH_{2n} peak whose intensity is greater than that of the corresponding C_nH_{2n} + 1 peak, when this fragment has a long straight chain tail. The even peak will be more intense than the odd peak when the fragment ion contains seven or more carbons in the chain. This phenomenon is not observed in a dialkyl substituted hydrocarbon when both substituents are located on the same carbon atom.

REFERENCES

1. T. Belsky, R.B. Johns, E.D. McCarthy, A.L. Burlingame, W. Richter and M. Calvin, Nature, 206, 446 (1965).
2. R.B. Johns, T. Belsky, E.D. McCarthy, A.L. Burlingame, Pat Haug, H.K. Schnoes, W. Richter and M. Calvin, Geochim. et Cosmochim. Acta, 30, 1191 (1966).
3. J. Oró, D.W. Nooner, Nature, 213, 1082 (1967).
4. J.M. Hayes, Geochim. et Cosmochim. Acta, 31, 1395 (1967).
5. H.C. Urey, Science, 151, 157 (1966)
6. M.H. Briggs and G. Mamikunian, Space Sci. Rev., 1, 674 (1963).
7. E.D. McCarthy, W. Van Hoeven and M. Calvin, Tetrahedron Letters, 4437 (1967).
8. D.W. Nooner and J. Oró, Geochim. et Cosmochim. Acta, 31, 1359 (1967).
9. J.M. Hayes and K. Biemann, Geochim. et Cosmochim. Acta, 32, 239 (1968).
10. E.D. McCarthy and M. Calvin, Nature, 216, 642 (1967).
11. E.D. McCarthy and M. Calvin, Tetrahedron, 23, 2609 (1967).
12. M.H. Studier, R. Hayatsu and E. Anders, Geochim. et Cosmochim. Acta, 32, 151 (1968).
13. K.E.H. Göhring, P.A. Schenck, E.D. Engelhardt, Nature, 215, 503 (1967).
14. J. Han, E.D. McCarthy, M. Calvin and M.H. Benn (A paper is in preparation).
15. K. Biemann, "Mass Spectrometry Organic Chemical Applications", McGraw-Hill, (1962), p.80.
16. G. Spiteller, "Massenspektrometrische Strukturanalyse Organisher Verbindunger", Verlag Chemie, (1966) P. 91.
17. J.P. Wibaut and H. Brand, Rec. Trav. Chim., 90, 97 (1961).
18. K. Biemann, "Mass Spectrometry Organic Chemical Applications", McGraw-Hill, (1962), Ch. 3.

19. J. Han, E.D. McCarthy, W. Van Hoeven, M. Calvin and W.H. Bradley, Proc. Natl. Acad. Sci., 59, 29 (1968).

20. W. Richter and D. Henneberg (private communication).

21. W. Van Hoeven. P. Haug, A.L. Burlingame and M. Calvin, Nature, 211, 1361 (1966).

22. G. Eglinton, P.M. Scott, T. Belsky, A.L. Burlingame, W. Richter and M. Calvin, in "Advances in Organic Geochemistry 1964", Eds. G.D. Hobson and M.C. Louis, Pergamon Press (1966).

23. J.G. Bendoraitis, B.L. Brown and L.S. Hepner, 6th World Petroleum Congress Proc. (1963), Frankfurt/Main.

24. James R. Maxwell, Ph. D. Thesis, University of Glasgow, Scotland (1967).

25. J.J. Cummins and W.E. Robinson, J. Chem. Eng. Data, 9, 304 (1964).

26. Emilio Gelpi, Ph.D. Thesis, University of Houston, Texas (1968).

27. A. Hood,"Mass spectrometry of Organic Ions," Ed. F.W. McLafferty, Academic Press, Inc., New York (1963).

28. R.J. Clerc, A. Hood, and M.J. O'Neal, Anal. Chem. 27 868 (1955).

29. S. Meyerson, J. Chem. Phys., 42, 2181 (1965).

30. S. Meyerson, Record. Chem. Progr. (Kresge-Hooker Sci. Lib.) 26, 257 (1965).

31. M. Kawal, Science and Culture, 30, 607 (1964).

CHAPTER 9 POSSIBLE EXTRATERRESTRIAL LIFE

1. Meteorites

An intensely bright fireball was observed over northern Mexico in the early morning of February 8, 1969 (01:09CST). The spectacular phenomenon was centered around the city of Parral in the South-Central part of the State of Chihuahua, the blue-white bolide having approached the area from south-southwest. The bolide broke into many pieces in the air near the small village of Pueblito de Allende, 35 Km. east of Parral. A piece of stone weighing about 15 kg fell within four meters of a house in that village. The total mass of the meteorite on entry into the atmosphere was estimated to be several tons.

Dr. E.A. King of NASA Manned Spaceflight Center in Houston left immediately for the scene and returned with approximately 6.8 kg on Feb. 12. B.R. Simoneit, Space Science Laboratory, University of California, Berkeley arrived in Parral on Feb. 15 and spent two days investigating the fall and collecting samples for research. Two pieces were brought back, one broken piece and another one which was used for research. That piece was approximately egg-shaped, weight 2.5 kg total with a fusion crust over its entire surface. A portion of 250 grams was obtained from Mr. Simoneit and used for organic analysis. The carbon content of the sample used in these studies was 0.35 $\pm$ 0.02%. King and his colleagues have published a report,[1] which shows it contained carbon to an extent of approximately 0.3%.

Clark et al.[2] reported the results of a comprehensive inorganic analysis. Visual inspection immediately suggested that this meteorite is a carbonaceous

chondrite, specifically a Wiik Type III[3]--it is closely similar to others of this type such as Grosnaja, Kaba, Lance, and Mokoia. Chemical and mineralological examination[2] confirmed this similarity. It has a Si/Mg ratio characteristic of the carbonaceous chondrites, and it falls within the range of other Type III meteorites. The conjection of iron-poor olivine in chondrules with iron-rich olivine in matrix is also distinctive for these meteorites. Yet the carbon content is low for a carbonaceous chondrite, lower in fact than in a number of chondrites whose Si/Mg ratio places them in the ordinary bronzite and hypersthene classes.

Organic analysis[4] was performed on the Allende meterorite here in Berkeley between March 1 and March 10, 1969. A 10 gram portion was used for a pyrolysis experiment. The surface of a 240 gram portion upon which the analysis was to be performed was first removed to a depth of about 1/4 inch, including all of the fusion crust as well as an approximately equivalent thickness of the fresh break which represented one surface. This operation, and all succeeding operations, was carried out in a clean cabinet through which a filtered air stream was continuously passing. The chips and center piece were analyzed separately, according to the flow sheet in Chapter 2.

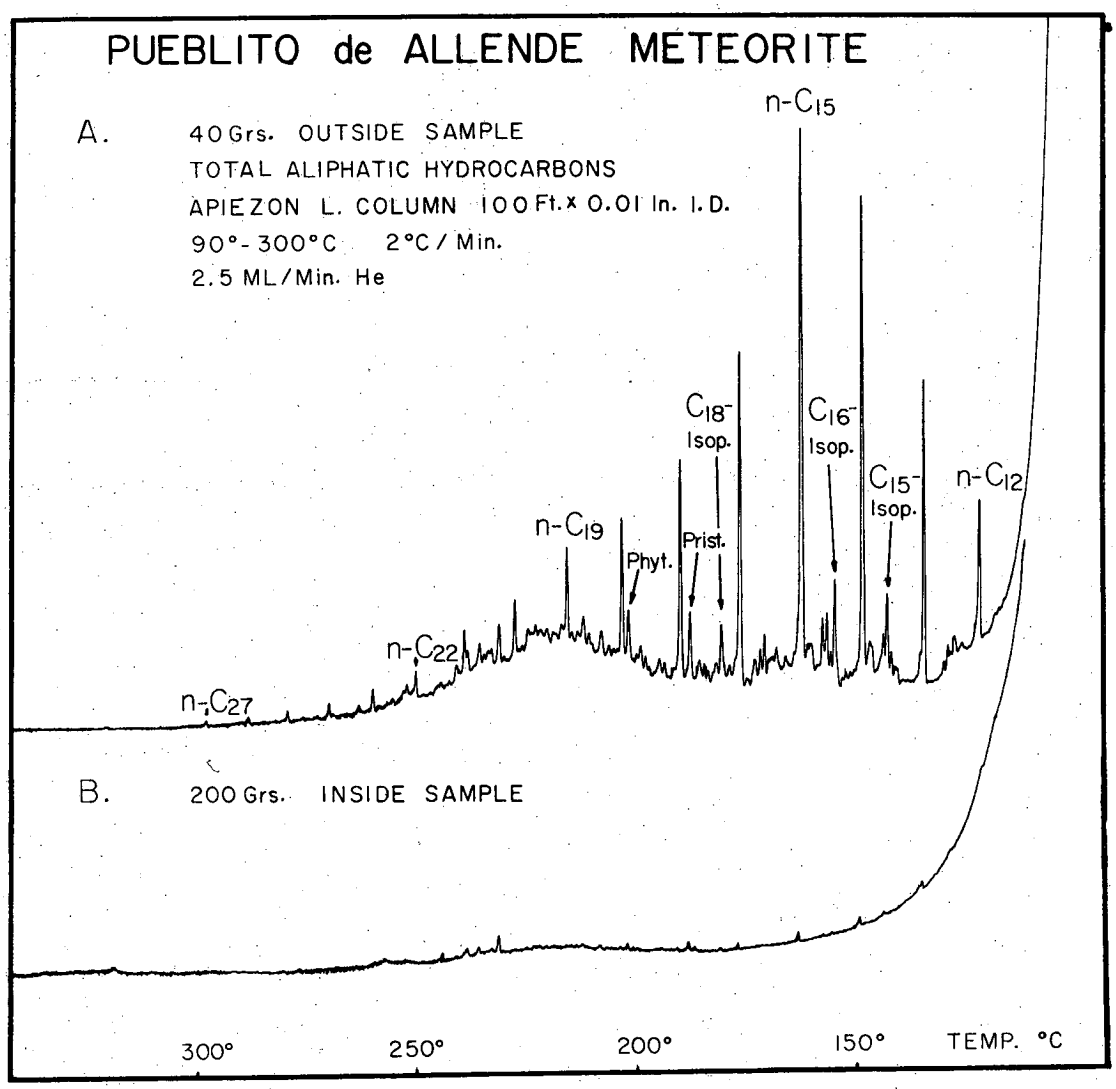
A. Total Aliphatic Hydrocarbons

The extractable organic compounds were separated into three fractions. All three fractions were examined chromatographically (Fig. 1,2,3) using a 100 ft. long and 0.01 inch inside diameter capillary column coated with Apiezon L stationary phase. The individual peaks were identified by their mass spectra. Combined gas chromatography-mass spectrometry was used for

Figure 1: Gas chromatogram of total aliphatic hydrocarbons from extractable organic material. Aerograph Model 204 gas chromatograph equipped with a flame ionization detector. About 1/10 of the n-heptane eluate was injected in each case.

A. 0.5 ppm of hydrocarbons were detected in outside sample.

B. 0.001 ppm of hydrocarbons were detected in interior sample.

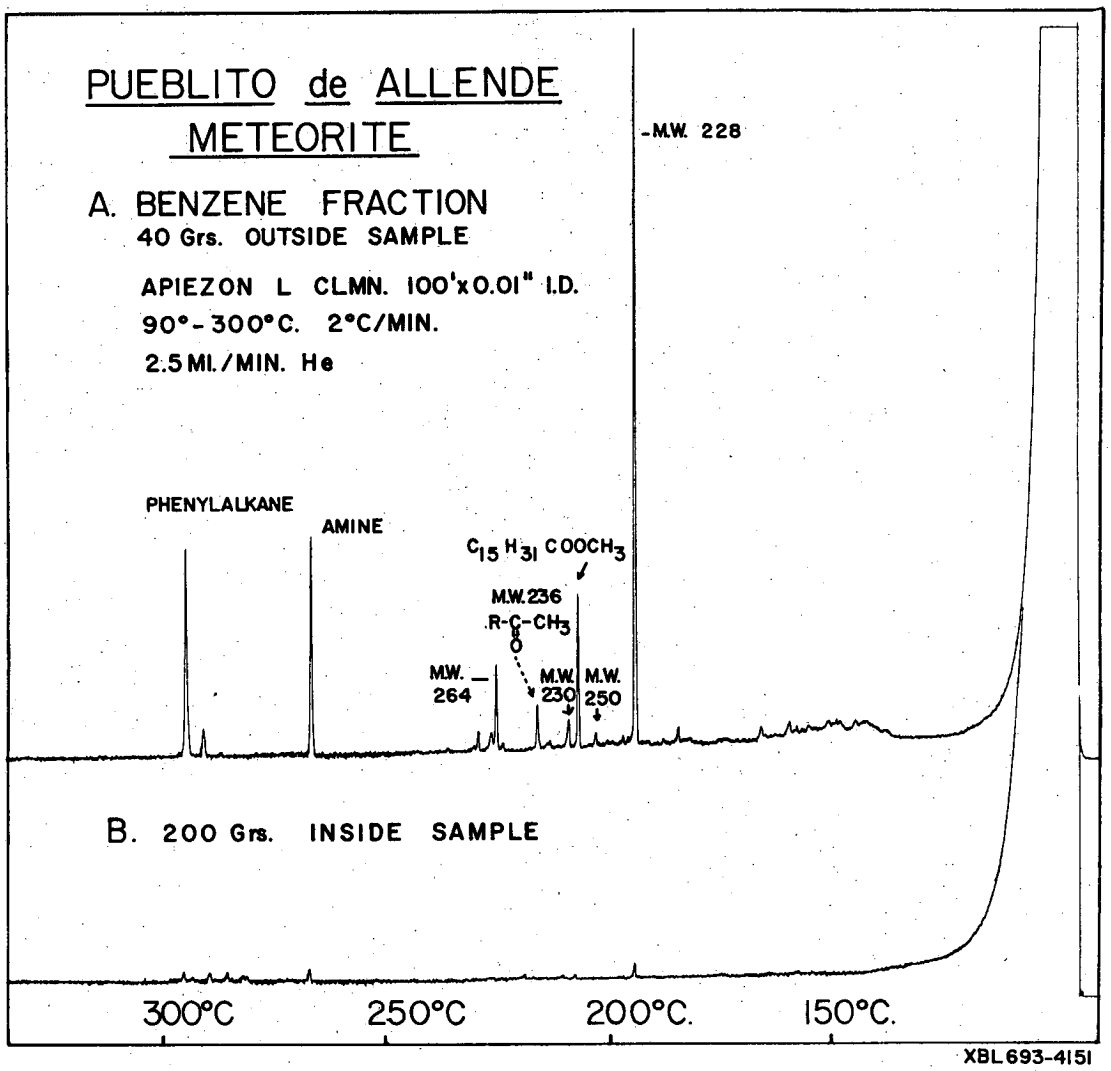

PUEBLITO de ALLENDE METEORITE
A.
40 Grs. OUTSIDE SAMPLE
TOTAL ALIPHATIC HYDROCARBONS
APIEZON L. COLUMN 100 Ft. x 0.01 In. I.D.
90°-300°C 2°C / Min.
2.5 ML/Min. He
n-C15
C18- Isop.
C16- Isop.
C15- Isop.
n-C12
n-C19
Phyt.
Prist.
n-C22
n-C27
B.
200 Grs. INSIDE SAMPLE
300°
250°
200°
150°
TEMP. °C

XBL 693-4146

Figure 2: Gas chromatogram of total benzene fraction from extractable organic material.

A. 0.2 ppm of organic compounds were found in outside sample.

B. 0.002 ppm of organic compounds were found in interior sample.

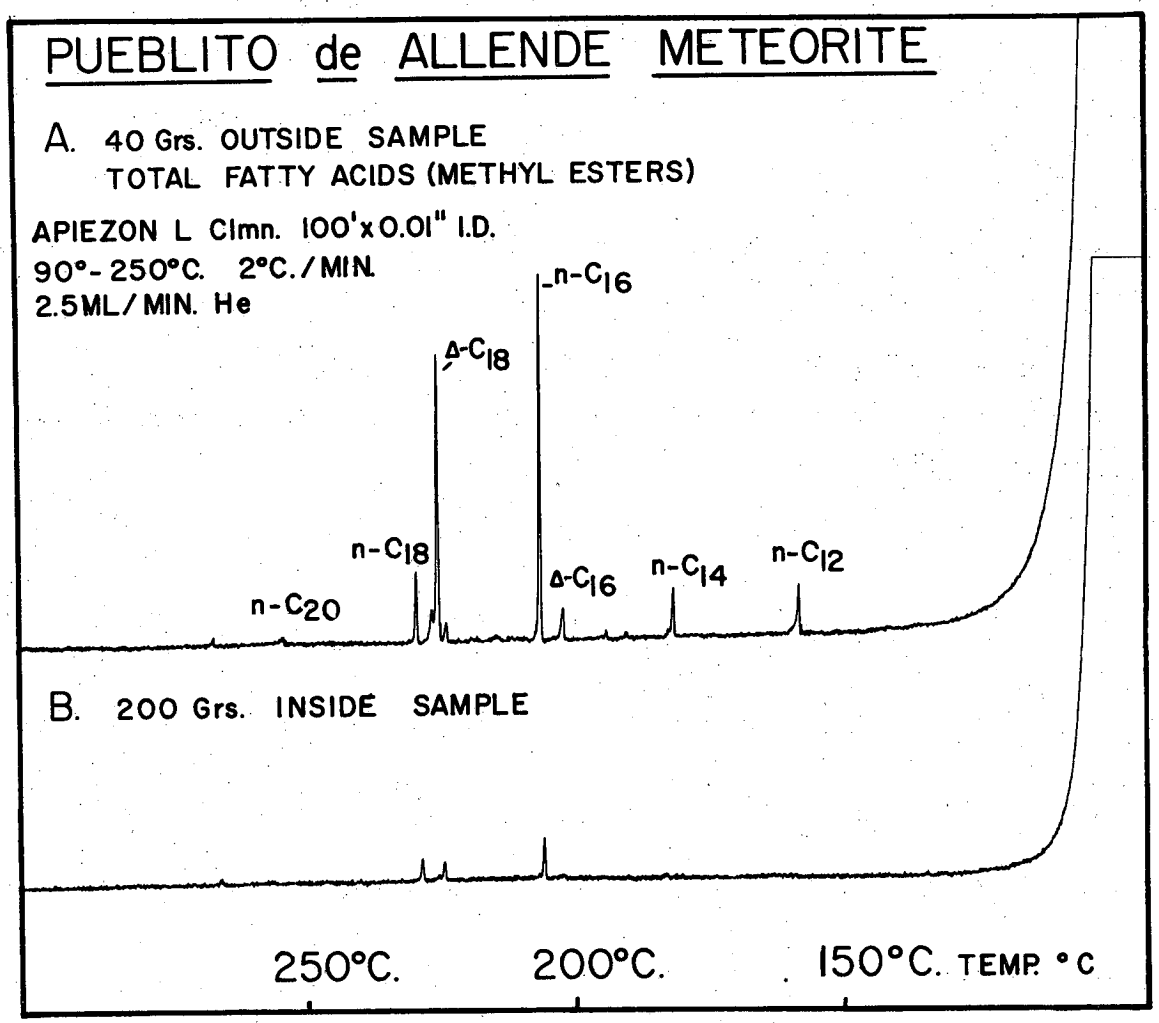

PUEBLITO de ALLENDE
METEORITE
A. BENZENE FRACTION
40 Grs. OUTSIDE SAMPLE
APIEZON L CLMN. 100'x0.01" I.D.
90°-300°C. 2°C/MIN.
2.5 ML/MIN. He
-M.W. 228
PHENYLALKANE
AMINE
$C_{15}H_{31}COOCH_3$
M.W. 236
$R-\underset{\underset{O}{\|}}{C}-CH_3$
M.W. 264
M.W. 230
M.W. 250
B. 200 Grs. INSIDE SAMPLE
300°C
250°C
200°C.
150°C.

XBL 693-4151

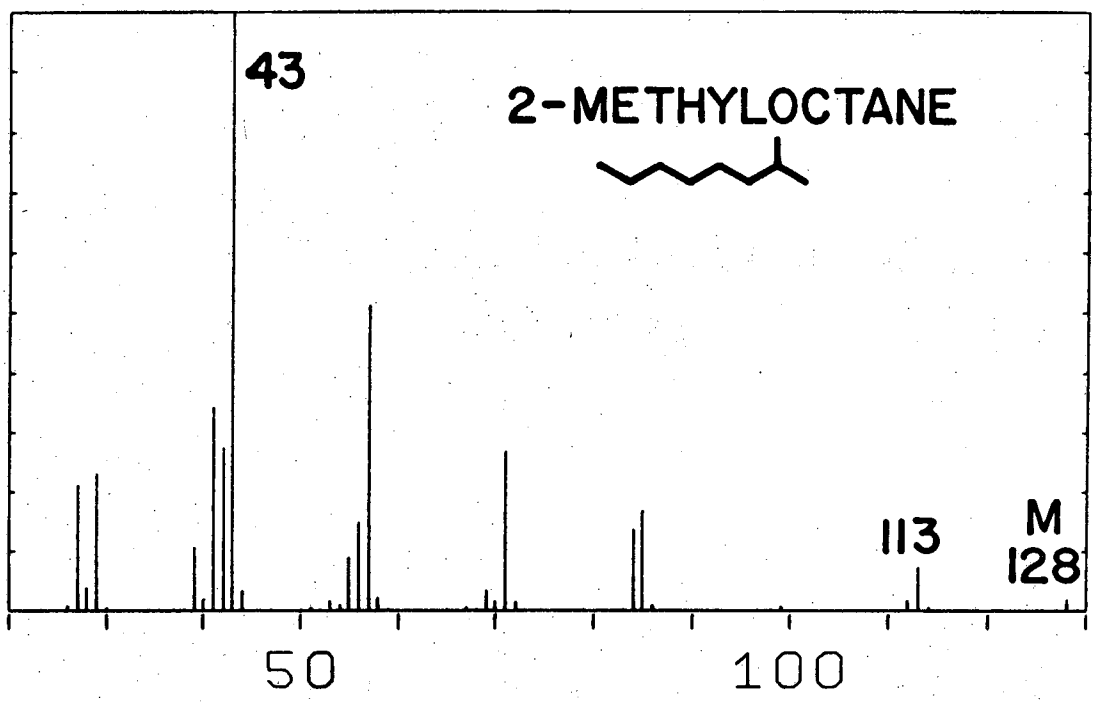
Figure 3: Gas chromatogram of total fatty acids (methyl esters) from extractable organic material.

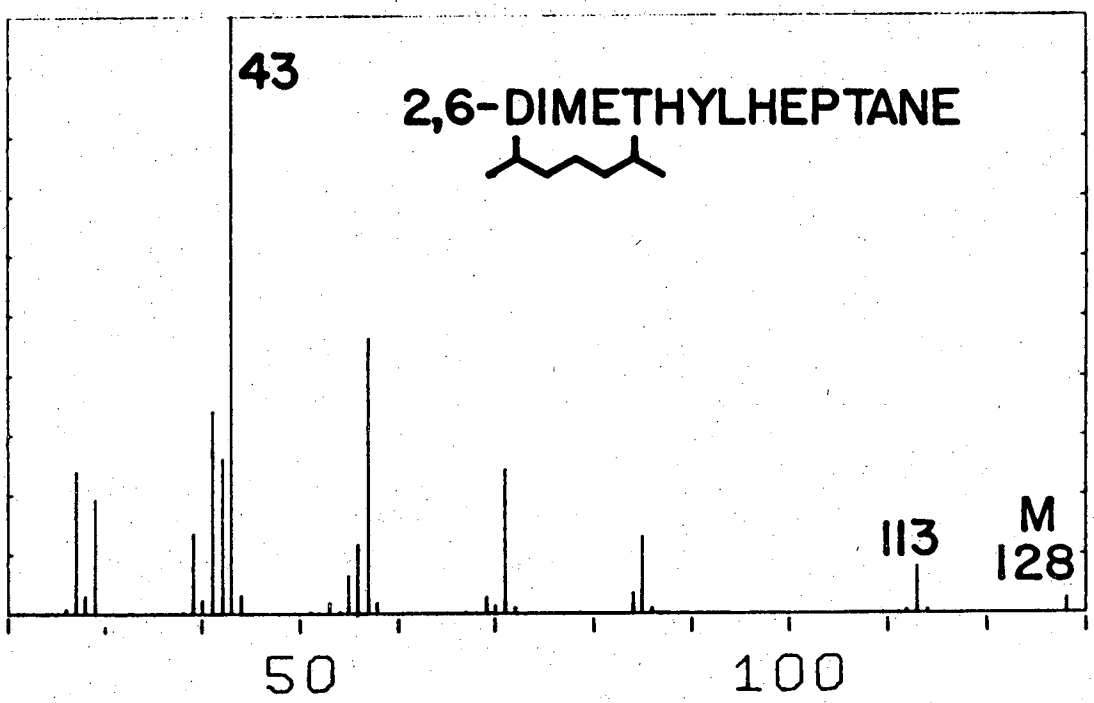
A. 0.1 ppm of fatty acids were detected in outside sample.

B. 0.001 ppm of fatty acids were detected in interior sample.


PUEBLITO de ALLENDE METEORITE
A. 40 Grs. OUTSIDE SAMPLE
TOTAL FATTY ACIDS (METHYL ESTERS)
APIEZON L Clmn. 100'x0.01" I.D.
90°-250°C. 2°C./MIN.
2.5ML/MIN. He
n-C16
Δ-C18
n-C18
n-C20
Δ-C16
n-C14
n-C12
B. 200 Grs. INSIDE SAMPLE
250°C.
200°C.
150°C. TEMP. °C

XBL693-4147





XBL 6711-6250

the structural investigation. Fig. 1A represents the chromatographic analysis of the total aliphatic hydrocarbons of the surface chips and the Fig. 1B chromatogram represents the analysis of the interior of the sample. Normal hydrocarbons from C_{12} to about C_{29} can be observed. Hydrocarbons of lower molecular weight do not appear in the chromatograms since these hydrocarbons would be volatilized by the evaporation to nearly dryness of the initial extracts and n-heptane eluates. Isoprenoid hydrocarbons from C_{15} to C_{21} except C_{17} are showing in Fig. 1. The surface chips contained 0.5 ppm of extractable hydrocarbons, while the interior sample only contained 0.001 ppm

Table 1 is taken from Oró and Nooner's report[5] which shows the isoprenoid and other aliphatic hydrocarbon contents in various meteorites. The Allende meteorite contained only 0.001 ppm of hydrocarbons in the interior sample which is more than three orders of magnitude lower than other Wiik Type III meteorites. This fact might be due to the short period of time of exposure in the field. The concentration gradient from outside to inside of the sample also suggests that at least the major part of the hydrocarbons are a result of terrestrial contamination. Since there is no odd over even predominance, and the C_{27}, C_{29}, and C_{31} peaks are not the major peaks, the contamination may come from the air or petroleum and not from plants or sediments.

B. Benzene Fraction

This fraction could contain conjugated alkenes, aromatic hydrocarbons, ketones, ethers, and other less polarized organic compounds. Fig. 2 shows the different type of organic compounds detected by GC. M.S. in this fraction.

The outside contains 0.2 ppm of organic compounds but the inside of the sample only contains 0.002 ppm. Again, the interior part is two orders of magnitude less than the outside chips.

C. Fatty Acids

As shown in Fig. 3, the Allende meteorite gives a chromatographic pattern in the C_{12} - C_{20} carbon number range with palmitic acid and oleic acid predominant. This pattern is similar to the fatty acid distributions of terrestrial living organisms.

It is quite clear that the organic material found in the surface layer of this meteorite is of biological origin and can hardly be other than terrestrial contamination acquired even in the short period of time available to the meteorite, namely, between its fall on February 8, 1969, and its collection on February 15, 1969. The speed with which this contamination was acquired and the diversity of the material contained in that contamination makes doubtful any interpretation of such organic materials as have been found in meteorites of unknown, or at least considerably longer, terrestrial history.[6-8] The presence of the mono-unsaturated C_{18} fatty acid in the Pueblito de Allende meteorite is additional confirmation of recent contamination. Such unsaturation has not been found in the Precambrian rocks.[9,10]

D. Bound Organic Compounds

The residue from the Allende meteorite, after extraction, was digested with a mixture of 1:1 HF:HCl, filtered off, washed with water, dried and repulverized. Extraction of the powdered residue with benzene and methanol as before afforded on extract containing organic compounds originally bound

to the inorganic matrix. This extract was separated and analyzed for aliphatic hydrocarbons, fatty acids, as well as benzene fraction. There was no detectable amount of organic material in the three fractions as shown by gas chromatography. A limit of o.1 part per billion (ppb) of possible indigenous organic matter can be detected by this method.

Other results in this group[9,10] have demonstrated that there are more bound fatty acids than the free ones found in the rock, if the acids have been there for a long period of time. Thus with very little doubt, the Allende meteorite is contaminated by terrestrial environment.

Exobiologists have studied the evidence for extraterrestrial life through for more than one century now. At this stage, we can conclude that the major part of organic compounds found in the meteorites are the result of terrestrial contaminations.

Now with the aid of NASA's program, scientists can start to make a giant leap--to study the extraterrestrial life on the moon, Mars or other planets, or bring the sample back to Earth for study in laboratories.

The first extraterrestrial exploration was accomplished on July 20, 1969, when Neil Armstrong made "a small step for a man but a giant leap for mankind."

2. <u>The Moon</u>

Man's eternal quest for the new and the unknown has led him to the highest mountains and the deepest ocean trenches, the most impenetrable jungles and the most forbidding deserts. Last year the bold attempt was successful. The journey to the moon will be remembered as long as the human race endures. It opened a new age of exploration, where man may travel

throughout the solar system, even the Universe, in flights, that could lead not merely to new worlds, new substances, new conceptions about the nature of matter and of life itself, but this exploration might discover extraterrestrial life, or might contact new intelligent beings as well.

A. Search for the early stages of chemical evolution of the Moon.

The search for organic compounds on the lunar surface is an important step in the understanding of the early stages of chemical evolution leading to the origin of life. In other words, the samples from the moon may give us some clues as to prebiotic organic synthesis in the solar system. The solar-wind flux of hydrogen, carbon, nitrogen and oxygen might be expected to result in the formation of organic compounds in the shallow surface layer of the exposed particles in the lunar fines and surface rocks.

The Apollo XI lunar material represents the first possibility of examining extraterrestrial material which has not been contaminated by terrestrial environment. An igneous origin has been reported[11] by the preliminary examination team for the surface rocks of the Sea of Tranquility. The fines consist chiefly of a variety of glasses, plagioclase, clinopyroxene, ilmenite, and olivine. Very rare spherules and rounded fragments of Ni-Fe up to 1 millimeter in size were observed. The glass, which constitutes about half of the material, is of three types: (a) botryfragments;

Table I

Isoprenoids and other Aliphatic Hydrocarbons in Meteorites

Group	Meteorite	Sample	Isoprenoids (p.p.m.)	Total* aliphatic hydrocarbons (p.p.m.)
Non-carbonaceous chondrites	Bruderheim (1)		0.02	0.8
	Chainpur (2)		0.13	2.8
	Indarch (3)		0.00	0.1
	" (3)		0.00	1.1
	Kainsaz (4)		0.12	2.7
	Karoonda (2)		0.11	3.2
	Average		0.06	1.8
Graphitic nodules from iron meteorites	Canyon Diablo (5)	outside	1.33	14.3
	" (5)	inside	0.22	2.8
	Odessa (6)	outside	1.18	15.5
	" (6)	inside	0.44	3.0
	Average		0.79	8.9
Wiik Type I carbonaceous chondrites	Alais (4)		0.33	5.9
	Ivuna (7)		2.42	22.6
	Orgueil (8)		0.72	9.0
	" (8)		0.00	7.8
	" (8)		0.08	4.2
	" (8)		0.04	10.0
	" (9)		0.38	25.0
	" (10)		0.45	4.6
	" (9)	outside	0.00	2.5
	" (9)	inside	0.00	0.1
	" (9)	"	0.02	0.3
	" (9)	"	0.04	2.4
	" (9)	"	0.12	1.0
	Average		0.35	7.3
Wiik Type II carbonaceous chondrites	Al Rais (7)		11.04	49.5
	Bells (7)		2.45	12.2
	Boriskino (7)		18.46	72.8
	" (4)		13.66	38.0
	Cold Bokkeveld(7)		6.79	46.0
	" " (3)		3.95	15.5
	Mighel (7)		24.18	127.7
	" (4)		4.97	27.8
	Murray (11)		4.61	35.8
	(4)		3.67	38.8
	Nogoya (7)		9.78	92.4
	Renazzo(12)		0.15	8.3
	(13)		0.24	8.9
	Santa Cruz (2)		35.44	150.0
	" " (2)		4.25	24.7
	Average		9.6	50.0

Table I (Cont'd)

Group	Meteorite	Sample	Isoprenoids (p.p.m.)	Total* aliphatic hydrocarbons (p.p.m.)
Wiik Type III carbonaceous chondrites	Felix (14)		3.15	128.0
	Grosnaja†(4)		3.04	415.0
	Kaba (4)		0.36	11.9
	Lance (13)		14.14	163.0
	Mokola (2)	replicate	7.75	29.3
	" (2)	replicate	6.69	33.3
	" (4)		3.71	15.8
	" (10)		6.40	32.3
	Ornans (4)		0.00	8.3
	Vigarano (14)		16.63	223.0
	" (10)		30.17	138.5
	Warrenton (4)		0.14	8.2
	Average†		8.1	72.0

(b) pale or colorless, or more rarely brown, yellow, or orange, angular fragments ranging in index of refraction from about 1.5 to about 1.6; and

(c) spheroidal, ellipsoidal, dumb-bell shaped, and tear-drop-shaped bodies, most smaller than 0.2 millimeter, which range in color from red to brown to green to yellow[11-15]. Reed[15] has noted that a proportion of the glass spheres appeared to contain vesicles as well as some optically dense inclusions. The inclusions within the glassy objects could be gas bubbles.

(i) Solvent Extraction--Extractable Organic Compounds.

A 19.8 gram sample of lunar fines was placed in a centrifuge tube and extracted with 10 ml 3:1 benzene:methanol for 30 minutes; an ultrasonic vibrator was used for this extraction. After the residue was removed, the solution was evaporated in five steps. At every step, approximately one microliter of solution was subjected to the gas chromatography-mass spectrometry system. In the final injection, which contained one third of the total extract, however, no response was observed on the recorder except for the solvent peak. The limit of detection has been shown to be 5×10^{-9} grams when a standard compound was analyzed by the system. Thus, the individual concentrations of extractable organic compounds were less than one part per billion.

(ii) Demineralization experiment--bound organic compounds.

The residue from solvent extraction was used for the demineralization experiment. Dissolution of the lunar sample was accomplished by slowly adding 20% hydrogen fluoride (250 ml) to a Teflon beaker which contained the dried residue. After two hours the digestion seemed to be complete, and the acid was removed with a centrifuge. The solid was washed three times with

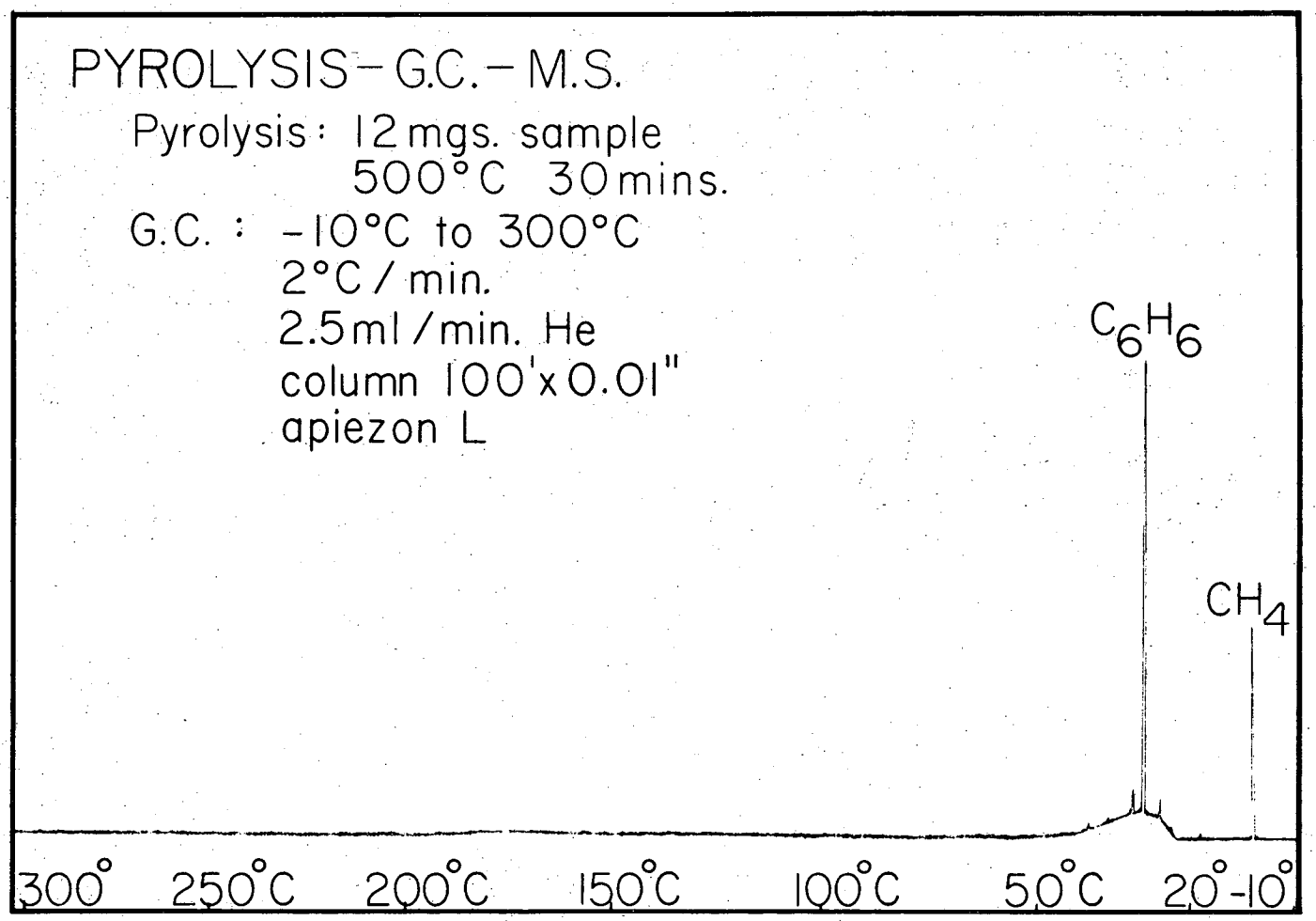
water to remove acid and fluoro-compounds. The resulting residue, weighing 12 grams, was mostly white floculent, with approximately less than one percent of heavy black particles. Both the acid and the residue were extracted with benzene. Benzene was removed stepwise from the combined extraction. For the final step, approximately one third of the extract was subjected to capillary gas chromatography. The bound organic compounds were not detected.

The preliminary results[11] obtained for the Apollo 11 rocks and fines point to an origin from high-temperature melts subsequently exposed on the surface under high vacuum for some millions of years. The presence of biogenic products is therefore extremely improbable, and the predominant sources of carbon are likely to be the solar wind and meteorite impact.

(iii) Pyrolysis--G.C.-M.S.

Pyrolysis of the lunar fines (12 mg) and demineralized residue (15 mg) was performed in a quartz, stopped-flow tube attached to a 3" x 1/8" silica gel trap which was connected to G.C.-M.S. system. The gas chromatographic column was a stainless steel capillary column 100 ft. x 0.01 in., Apiezon L; the temperature was programmed from -10° to 300°C. The sample was preheated at 150°C for 15 minutes to remove absorbed material before the valve of the pyrolyzer tube was closed. After it was heated at 500°C for 30 minutes, the valve of the pyrolyzer was opened and (20 psi) of helium gas swept all the volatile compounds to the silica gel-liquid nitrogen trap. Multiple mass scans were obtained of the volatile compounds released by heating of the silica gel trap. Fig. 4 shows only two major products which represent methane and benzene. However, methane was the only product for the pyrolysis of the demineralized sample.

Figure 4: Gas chromatographic separation of pyrolysis-G.C.-M.S. analysis of lunar dust from the Sea of Tranquillity. 12 mgs of sample was used at 500°C pyrolysis temperature for 30 mins. A stainless steel tubing 100 ft. x 0.01 in. coated with Apiezon L. Helium pressure, 20 psi. Temperature programmed at 2°C a minute from -10°C to 300°C.


PYROLYSIS-G.C.-M.S.
Pyrolysis: 12 mgs. sample
500°C 30 mins.
G.C.: -10°C to 300°C
2°C/min.
2.5 ml/min. He
column 100'x0.01"
apiezon L
C_6H_6
CH_4
300°
250°C
200°C
150°C
100°C
50°C
20°
-10°

XBL705-5239

(iv) HF treatment--bound gas analysis.

The optical data[16-19] showed that the glass spheres contained vesicles as well as some optically dense inclusions. The inclusions could be trapped in the lunar fines. Hydrogen fluoride was used to dissolve the dust and gases could be liberated. The schematic of the apparatus used is shown in Fig. 5. The system was made of glass with Westef stopcocks and Cajon trap disconnectors. In order to check the recovery efficiency of the system a silica gel blank was run. The whole system was alternately purged with helium three times and pumped down before the flask was doped with 1 ml of CO. The flask was valved off and stirred during the stepwise addition of 150 ml HF. The traps were under vacuum and the respective temperatures of the coolants. After purging all the volatiles from the flask into the dry ice-acetone trap with helium, the flask again was isolated. By a series of heating and freezing cycles, the volatiles were transferred to the liquid helium trap and valved off. The helium trap pressure was measured and the contents analyzed by high resolution mass spectrometry, (A.E.I. MS-9). The 17.8 grams of lunar fines were degassed at 150°C for 15 minutes in the same system under a helium atmosphere. The same procedure was followed as described for the blank run.

The recovery efficiency of this system was found to be 50%. A total of 66 ppm carbon was recovered as CO from the liquid helium trap. The presence of methane, neon and argon was also indicated.

It appears that the largest chemical component form of the carbon which is present in the lunar fines is carbon monoxide. This could be the result of the oxidation of any reduced carbon by the heat of meteorite impact or

other source of energy available to the lunar surface, using mineral oxides as the oxidizing agent. During the meteorite impact, the lunar surface temperature could be higher than 1200°C, and this oxidation reaction would be proceeded. Most of the carbon monoxide would have escaped the lunar gravitational force, thus accounting for less than 0.02% of total carbon in any of the samples yet analyzed.

(v) Possible biogenic compound on the Moon.

From the radio waves measurement, the density of gases on the moon is at least one trillion (10^{12}) times less than on the surface of the Earth. The absence of atmosphere implies total dryness, since it is inconceivable that water could exist in the liquid state in so high a vacuum. We must, therefore, accept the fact that the lunar surface is a desert and that there are no seasons in the climatic sense. The measurements on the Apollo 11 samples have shown that in the regions of the moon where the sun reaches the zenith, the surface temperature is as high as 130°C. This value falls as the sun declines towards the horizon and at sunset reaches 60°C. During the long lunar night, which lasts for the equivalent of two earth weeks, very considerable cooling of the surface occurs, in the absence of any protecting atmosphere, and the ground temperature drops to -80°C and even to as low as -150°C. For this surface condition, extraterrestrial life could never exist, and the process of chemical evolution would be unable to operate.

At a depth of roughly 50 meters below the lunar surface, comfortable temperatures, by our standards, can be expected--temperatures which remain constant through the lunar day and night. At similar depths, several astronomers and geologists have suggested, there should be subsurface water

which is prevented from escaping to the surface by an overlying layer of ice, in exact analogy to the permafrost of the terrestrial arctic and antarctic regions. If there is a region below the surface of the Moon which has warm temperatures, liquid water, and the possibility of primitive organic matter, then it seems premature to exclude the possibility of life on the Moon. However, indigenous life seems highly unlikely because there is no energy source besides the radiation energy and chemicals locked in the subsurface materials and the organic matter. This energy is limited at best. If life once arose at such lunar depths, it would occur only in a brief period of time.

3. Mars

Mars is the only planet in the solar system other than the Moon whose surface features have been photographed and measured by Mariner 6 and 7 under telescopic observation. Observers have long suspected that the expansion and contraction of the dark areas of Mars indicate vegetation of some sort. The dark areas expand at a rate of 15Km per day in the Martian spring following the melting of the ice cap. Many studies of Mars support the hypothesis that some sort of vegetation is present.[20] If this proves to be correct it will be of the utmost interest to determine whether or not this life is based on nucleic acids and proteins.

Life as we know it depends on the availability of water and carbon dioxide among other things, and only recently these have probably been conclusively identified by infrared spectroscopy in the Mariner 6 and 7 experiments. Mars has an atmosphere, but it is much more tenuous than that of the earth, and of different composition. The pressure of the atmosphere at the Martian surface is believed to be 25 $\pm$ 15 millibars. At the equator,

the temperature may vary from 30°C to -80°C between noon and midnight. It is obvious, therefore, that organisms exposed at the surface of Mars would be frozen. Some anaerobic bacteria can withstand treatment of this sort, but it is also well known that remarkable degrees of resistance to cold have been found among quite complicated animals.

Mars might easily support life forms and the flexibility of organisms we know may be far greater than is generally believed. If Mars has no life of its own, the colonization of Mars by terrestrial organisms could be attempted.

Speculation concerning the existence of extraterrestrial life has always intrigued a small segment of the scientific community. However, only during the last few years has this type of speculation taken practical meaning. Within a few years Viking mission will obtain scientific data about Mars, with particular emphasis on providing information relevant to life on the planet.

4. The Universe

A fundamental process affecting the prevalence of life in our universe is the formation of planetary systems. The evolution of biological life following a period of chemical evolution requires a suitable and stable physical environment which may exist on some planets. It may therefore be expected that the prevalence of life in our universe would be directly proportional to the number of existing planetary systems, whether there are few or many, depends on how they are created.

Theories of planet formation may be classed as collision formation theories and natural formation theories. Theories of the former type generally imply that planet formation is an extremely rare occurrence and

therefore the development of biological life must be a rare phenomenon. However, the natural formation theories imply the existence of large numbers of planetary systems and, therefore, frequently occurring environments suitable for chemical and biological evolution.

CHAPTER 9 REFERENCES

1. E.A. King, E. Schonfeld, K.A. Richardson and J.S. Eldridge, Science 163, 928 (1969).

2. R.S. Clard, E. Jarosewich, B. Mason and J. Nelson, The Allende Meteorite, A Preliminary Report, Smithsonian Inst., Washington D.C. (1969).

3. H.B. Wiik, Geochim. Cosmochim. Acta 9, 279 (1956).

4. J. Han, B.R. Simoneit, A.L. Burlingame and M. Calvin, Nature, 222, 364 (1969).

5. J. Oró and D.W. Nooner, Nature 213, 1085 (1967).

6. J.M. Hayes and K. Biemann, Geochim. Cosmochim. Acta 32, 239 (1968).

7. M.H. Studier, R. Hayatsu, and E. Anders, Ibid. 32, 151 (1968).

8. D.W. Nooner and J. Oró, Ibid. 31, 1359 (1967).

9. W. Van Hoeven, J.R. Maxwell and M. Calvin, Ibid, 33 , 877 (1969).

10. J. Han and M. Calvin, Nature 222, 576 (1969).

11. Lunar Sample Preliminary Examination Team, Science, 165, 1211 (1969).

12. P. Cloud, S.V. Margolis, MV. Moorman, J.M. Baker, G.R. Licari, D. Krinsley, and V.E. Barnes, Science 167, 776 (1970).

13. A.T. Anderson, A.V. Crewe, Glodsmith, J.R., P.B. Moore, J.C. Newton, E.J. Olsen, J.V. Smith and P.J. Wyllie, Science 167, 587 (1970).

14. J.C. Bailey, P.E. Champness, A.C. Dunham, J. Esson, W.S. Fyfe, W.S. MacKenzie, W.E.F. Stumpfl, J. Zussman, 167, 592 (1970).

15. W. Reed, Ph.D. Thesis, Department of Geology, Univ. of Calif. Berkeley. (1970)

16. J.J. Grossman, J.A. Ryan, N.R. Mukherjee, M.W. Wegner, Science, 167, 743(1970).

17. A.L. Burlingame, M. Calvin, J. Han, W. Henderson, W. Reed, and B.R. Simoneit, Science, 167, 751 (1970).

18. S.W. Fox, K. Harada, P.E. Hare, G. Hinsch, and G. Mueller, Science, 167 767 (1970).

19. B. Nagy, C.M. Drew, P.B. Hamilton, V.E. Modzeleski, M.E. Murphy, W.M. Scott, H.C. Urey, and M. Young, Science 167, 770 (1970).

20. S.L. Hess, Adv. in Space Sci. and Tech, 3, 151 (1961).

CHAPTER 10 CONCLUSION

The present work might be divided into two main parts dealing with 1) the living organisms, 2) the non-living systems such as sediments, petroleum, meteorites, and the lunar samples. The former part contains Chapters 4-7 while the latter one is illustrated in Chapters 3, 8 and 9. The living organisms were studied on the basis of chemical taxonomy and comparative biochemistry while the non-living systems were evaluated by their molecular fossils and biological markers. However, these two parts are closely related; one could not study the one without having the knowledge of the other. In order to learn the nature of the organic compounds that might have existed in microorganisms billions of years ago, we also need to know more exactly the nature of the material we are starting with today, so that we can make some estimates of what to expect as we go back in time. This leads to a comparative examination of organic structures that can be found in today's microorganisms. This same type of knowledge is also essential for examining extraterrestrial materials. There is only one way we know, at the present time, which extraterrestrial forms of life originated independently of life on the Earth. That is by comparing the skeletons of organic compounds of the extraterrestrial life with that of life on the Earth. This suggests that the life as we know it carries a trade-mark of some kind, one that is revealed by chemical taxonomic analysis that says in effect "made on Earth". Micropaleontologic evidence may help us to decide the evolutionary steps of microorganisms, which knowledge is essential for comparative biochemistry studies.

This thesis also could be divided another way--all the chapters preceding Chapter 9 are the chemical studies of terrestrial life and their diagenetic products in sediments. Chapter 9 discusses the search for extraterrestrial life. This work is only a beginning, and we are looking forward to see some constructive development in the near future.

Modern analytical techniques have been applied to these studies. The combination of gas chromatography and mass spectrometry especially has made possible the detailed study of the individual components of complex organic mixtures regardless of their origin. Synthetic work has been carried out by means of structural conformational studies as well as synthesizing compounds of authentic structure. The biosynthetic pathway of normal and branched hydrocarbons in the blue-green algae has been studied by using carbon and hydrogen isotopes as tracers. The study has also revealed much of the biological order which prevails at the molecular level, and as a consequence, useful criteria of biogenicity have been developed on the basis of the results obtained throughout this investigation.

The methyl-branched alkanes seemed to be of particular significance in blue-green algae because the methyl branches are not iso- and anteiso-structures. They appear to occur uniquely in blue-green algae and are absent from photosynthetic and non-photosynthetic bacteria. These hydrocarbons have not been found in the green algae, which are morphologically more advanced than blue-green algae but are still relatively primitive. The branched C_{18} compound was elucidated by organic synthetic work to be 1:1 7- and 8-methylheptadecane. It was finally separated by 750 ft. of high resolution capillary column, which

showed that 10% of 6-methylheptadecane is also present in the mixture. This branched hydrocarbon was proved to be optically active; the activity $+2.5^{\circ} \pm 0.5$ was obtained. The gas chromatographic evidence shows that the branched C_{18} was present in Soudan Shale. Thus Barghoorn's morphological microfossil evidence was confirmed. The biosynthetic pathway to this 50:50 mixture of 7- and 8-methylheptane was proved to be the methyl group insertion to cis-vaccenic acid, followed by decarboxylation reaction.

The question now arises as to the diagenetic fate of terrestrial and extraterrestrial organic matter which once has been synthesized on a prebiotic earth or on the planets. Its occurrence can be inferred by the presence of kerogen, i.e., carbon in ancient sediments and extraterrestrial materials, as old as 3-5 billion years. The elucidation of the diagenetic history of this material may reveal important details concerning the origin of life on earth and perhaps elsewhere in the universe.

Over the past three decades, much has been learned of a quantitative nature of fossil organic matter present in various terrestrial and extraterrestrial rock materials. These studies have evolved far beyond the estimation of total organic carbon and nitrogen to the isolation and measurement of specific kinds of organic matter, for example, amino acids, carbohydrates, heterocyclic compounds and lipids.

In the early organic geochemical studies, the presence of sugars, amino acids, and bases of purines and pyrimidines in hydrolysis liquors of ancient rocks were used as indications that intact polysaccharides, polypeptides,

nucleic acids, and other biogenic polymers could survive diagenesis. The present consensus, however, is that during diagenes new polymers, or condensates, are synthesized from breakdown products of former biochemical macromolecules. Namely, the bulk of the fossil organic matter has acquired its molecular framework in the course of diagenesis, whereas biogenic macromolecules, such as proteins or nucleic acids, are eliminated rather radically in the early stages of diagenesis.

The research on hydrocarbon and fatty acids constituents of sediments has attained a sufficient level of sophistication that it is possible to state whether or not those compounds are the result of biological activity. Whether the existence of organic compounds in the Onverwacht, the oldest sediment known as earth, upon which these experiments have been performed can or cannot be regarded as evidence of biological activity is still questionable. Since the rock is porous enough to let water pass through, after several billion years, it is not surprising if one would find organic compounds in the parts per million range.

The finding of C_{22} to C_{25} isoprenoids in ancient sediments proved that the precursors of isoprenoid hydrocarbons are the alcohols, such as farnesol, phytol, betulaprenols, solanesol, etc., but not the hydrocarbons, squalene and lycopene. The initial degradation process had to take place by attack at the alcohol end, followed by reduction and thermal cracking mechanisms to give isoprenoid hydrocarbons found everywhere in terrestrial materials.

In mass spectrometry, branched alkanes are identified by their molecular ion and by their characteristic cleavage at branched positions. Another

characteristic fragment, the even mass numbered peak due to the hydrogen atom transfer, can be used as important information to interpret a mass spectrum. The fragmentation of a saturated aliphatic hydrocarbon would cause a hydrogen transfer giving rise to a $C_nH_{2n} + 1$ peak, when this fragment has a long straight chain tail. The even peak will be more intense than the odd peak when the fragment ion contains seven or more carbons in the chain.

The mass spectrum of olefinic hydrocarbon for example shows a relatively high M-28 ion; this might indicate that an ethylene molecule is split out from its long tail end.

The identities of methyl branched fatty acids can be easily deduced mass spectrometrically from the presence of fragments corresponding to the formation of a ketone ion, derived from the branched structure through the loss of a terminal alkyl and methanol and the loss of water from the ketene ion.

The n-C_{17} hydrocarbon is predominant in algae. Its dominance is decreased in photosynthetic bacteria, and it is not outstanding in other living organisms. The isoprenoid hydrocarbons are generally absent in algae, but they are generally present everywhere else in terrestrial living and non-living materials.

The nature, distribution, quantity and concentration gradient of the organic compounds found in Allende meteorite indicates that those molecules are terrestrial contaminations. Thus it suggests we cannot study extraterrestrial life without ambiguity through meteorites.

The study of lunar surface samples neither shows signs of extraterrestrial life nor indicates that chemical evolution has progressed on the surface

of the moon. This is a good beginning toward the search for extraterrestrial life, and this search will never be stopped as long as the human race endures. We did not find extraterrestrial life on the moon; we might not find it on Mars or Venus. However, some time, someday, as our exploration goes on, we not only might find a single cell somewhere in the universe, but also find another being as well.

I should like to end this thesis by referring to MacGowan and Ordway's words:

> "Man was created on, evolved on, and today has become master of a tiny speck of dust adrift in a vast sea of blazing suns. As he matured, he patiently studied the myriad manifestations of nature and sought to organize what he observed into a comprehensive unified synthesis of his newly won knowledge. This, he hoped, would illuminate the path towards certain universal fundamentals he felt confident are as valid to worlds infinitely far removed, as they are to his home planet, Earth."[1]

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1. R.A. MacGowan and F.I. Ordway, "Intelligence in the Universe," Prentice-Hall, Englewood Cliffs, N.J. p. ix (1966).