PYROLYSIS OF SOME FATTY AND AMINO ACIDS

A Thesis

Presented to the

Faculty of the Department of Chemistry

College of Arts and Sciences

University of Houston

In partial fulfillment

of the requirements for the degree

Master of Science

by

F. Edward O'Neill, Jr.

August 1967

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ABSTRACT

Myristic, palmitic and stearic acid were pyrolyzed at 300°C for two hours. In addition to olefins, straight-chain alkanes were found having a carbon number of up to 6 greater than the original fatty acid. The predominant n-alkane is the one that results from the decarboxylation of the fatty acid. Pyrolysis under pressure as well as increased heating time were not found to significantly improve the transformation of the fatty acid to aliphatic hydrocarbons. Mixed fatty acids and the methanol fraction of a bacterium were pyrolyzed and gave products which were a predictable result of the decarboxylation of the parent compounds, as well as additional hydrocarbons.

Phytanic acid was pyrolyzed and gave predominantly branched alkanes. The decarboxylation product, pristane, was not obtained in major yield, but some straight chain alkanes were produced. These results are compared to the hydrogenolysis and thermal degradation of phytol, which gave important yields of pristane and phytane.

Two amino acids were pyrolyzed, L-leucine and L-alanine. No hydrocarbons were produced and it was concluded that amino acids are not an important source of petroleum hydrocarbons.

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INTRODUCTION

INTRODUCTION

Fatty Acid Pyrolysis

I. Definition

> "Pyrolysis may be defined as the conversion of one substance into another by the agency of heat alone, or heat with the aid of a catalyst (1)."

Pyrolytic reactions generally take place in an inert atmosphere or a vacuum. The products formed are a result of factors which entail degradation, decarboxylation, polymerization and dehydration. By controlling extrinsic factors such as temperature, catalyst and heating time, the type and amount of products formed may also be controlled. In practice, the term "pyrolysis" is frequently used to apply to reactions in which multiple reactants, pressure, air or other substances are used whose influence on the reaction is unknown. These add new dimensions to the pyrolytic reactions, which may occur alone, simultaneously, or in sequence.

Fatty acids are monobasic organic acids derived from natural fats and oil. Those acids whose molecules have an even number of carbon atoms arranged in a straight chain are by far the most common and may be either saturated or unsaturated. The most abundant are those with 16-18 carbon atoms (2).

II. Scope

Although fatty acids have been found to yield a variety of compounds on thermal decomposition, the picture has been somewhat complicated by

the large number of different conditions employed. Table I presents a summary of some of the products obtained under varying conditions that are of interest. Of these, the principal products seem to be ketones, olefins and paraffins. It is the latter with which this thesis is mainly concerned. In many cases, paraffins were not reported and it is felt that examination for paraffins was not made, or that they were of no particular interest to the author.

III. Historical

Probably the oldest and most well known reaction involving pyrolysis of fatty acids is the preparation of ketones by heating the calcium or barium salts or fatty acids (14). However, as early as 1856, an equally important decarboxylation pyrolysis was found to produce various hydrocarbons (15). Both reactions have been utilized in the production of fuel; the latter being principally investigated to this effect, and they are especially important in countries such as China and Japan where natural supplies of petroleum are lacking, but which contain large amounts of fatty acids, both saturated and unsaturated, in the form of animal fats, vegetable oils, and other natural sources (9,16-23). Table II lists some of the products obtained from mixed fatty acid sources. The success of these processes have been reflected in the numerous patents which have been registered since 1930.

The decarboxylation reaction of free monobasic fatty acids to produce the corresponding paraffin is the most general pyrolytic reaction (24)

TABLE I

INDIVIDUAL FATTY ACID AND SALT PYROLYSIS

Reactant	Conditions	Products	Ref.
Stearic acid	Tin and lead catalysts, 400°C, 3-5 hours	heptadecene heptadecane	3
Stearic acid	Iron catalyst, 20 hours	crude olefins	4
Stearic acid	Iron catalyst, 360°, 4 hours	stearone	5
Stearic acid	280-290°, 80-90 mm Hg, 4 hours	corresponding ketone, anhydride and carbohydrate	6
Sodium stearate	Dry distillation	decane tetradecane pentadecane tetracontane	7
Calcium stearate	Distillation, 12-15 mm Hg	decane tetradecane pentadecane tetracontane	8
Calcium stearate	Iron retort, 315-540°	paraffins naphthenes olefins aromatics	9
Hexanoic acid	Anhydrous, 220-300° 50-60 hours	caprone pyrones	10
Octanoic acid	Silica with 10% Al catalyst	heptene ketones CO_2 olefins	11
Myristic acid	400°, 250 atm, 6-8 hours	paraffins haphthenes	12
Behenic acid	Bentonite clay, water 200°, 89 hours	n-alkanes	13

TABLE II

PYROLYSIS OF MIXED FATTY ACIDS

Acids or Source	Conditions	Products	Ref.
Calcium stearyl Palmitate	220°, 8 hours, distillation	Paraffins	28
Coconut, palm, and chrysalis oils	mixed clay, metal oxides, 450°C	Predominantly paraffins	19
Soybean oil, calcium and magnesium salts	450°C, several hours	Heptane, octane, nonane other paraffins	17,18
Tung oil, calcium salts	315-540°C	Aromatics, olefins, paraffins	30
Pork fat Coconut oil	300-340°C	12-pentacosanone	31
Coconut oil	Activated acid clay 400-500°, 40 minutes lime	C_8-C_{16} olefins	32
	BaO	Saturated hydrocarbons, aldehydes, ketones	33
Shark liver oil	$AlCl_3 \cdot 6H_2O$	Olefins, ketones	20

$$RCO_2H \rightarrow R\text{-}H + CO_2 \qquad (1)$$

Piria (15) first reported obtaining hydrocarbons by heating the fatty acid salts with a metallic hydroxide. Although during the latter half of the nineteenth century there was a prevalent idea that petroleum was ultimately derived from the fats of animals (25), it was Engler (26,27) who first popularized it, incorporating his results of heating fats from various animal sources to 360-425° under 4-10 atmospheres of pressure. In this series of experiments, he obtained liquid hydrocarbons resembling natural petroleum and proposed that these fats were first hydrolyzed by water and subsequently subjected to the effects of pressure and heat in nature (29).

Kunkler (28) observed that on heating oleic and steric acids under atmospheric pressure and temperatures somewhat below their boiling points, the formation of petroleum-like hydrocarbons containing a measureable amount of ketones was obtained. Pictet and Potok (8) developed Engler's theory by taking the individual acids, and distilling them under partial vacuum. From sodium stearate he obtained almost exclusively paraffins, notably decane, tetradecane, pentadecane and tetracontane. From calcium oleate, he obtained the corresponding monounsaturated compounds. This work substantiated the observation of Kunkler that ketones act as intermediates in the formation of hydrocarbons from fatty acids. Under milder conditions, Zelinsky and Lawrowsky (34) obtained liquid paraffins from distillation of palmitic and stearic acids. Petrow (12), by applying pressure to the pyrolysis of myristic acid, obtained naphthenes as well as paraffins.

After the work of these early investigators, until about 1950, few fatty acid pyrolyses to saturated hydrocarbons have been examined. Most work has been centered around unsaturated products, mainly because of their potential use as lubricating oils. However, few saturated fatty acids have produced olefins in high yields (1). Since it has been established that ketones are intermediates (8, 28), any decarboxylation to olefins might also be expected to concommitantly give paraffins.

In 1950, calcium stearate was pyrolyzed from 315-540°C (9) and the cracked distillate yielded 24.8% naphthenes and paraffins. A proposed mechanism presumes that the pyrolysis follows the successive steps: dehydration to the anhydride, decarboxylation to saturated ketones, and decarboxylation to paraffins and olefins (1).

$$2(R\text{-}COOH) \xrightarrow{\Delta} \begin{matrix} R\text{-}C(=O)\text{-}O\text{-}C(=O)\text{-}R \end{matrix} + H_2O \qquad (2)$$

$$2\ \begin{matrix} R\text{-}C(=O)\text{-}O\text{-}C(=O)\text{-}R \end{matrix} \xrightarrow{\Delta\Delta} 2\ R\text{-}C(=O)\text{-}R + CO_2 \qquad (3)$$

$$2R\text{-}C(=O)\text{-}R + H_2O \xrightarrow{\Delta\Delta\Delta} 2RH + CO_2 \qquad (4)$$

This scheme does not account for the naphthene, olefinic and aromatic hydrocarbons produced, but has been subsequently verified by techniques using isotopically labeled C-14 in the carbonyl position of acetate (35,36). These same authors also gave evidence for a free radical R· which results

from homolytic cleavage of the R-COOH bond. Although it was possible that another mechanism might ensue (37), Reed confirmed that at least in the decarboxylation to a ketone, the reaction proceeds by way of a free radical mechanism (38).

The existence of free radicals in the pyrolytic decarboxylation of fatty acids now made it possible to account for the presence of paraffins with a higher number of carbon atoms than the parent fatty acids that were sometimes observed among the products. An example would be tetracontane, produced by Pictet and Potok from the pyrolysis of calcium stearate. The overall reaction involving a ketone intermediate is given in two steps

$$2C_{17}H_{35} - CO_2H \rightarrow 2C_{17}H_{35} - \overset{O}{\overset{\|}{C}}-C_{17}H_{25} + CO_2 + H_2O \qquad (5)$$

$$2C_{17}H_{35} - \overset{O}{\overset{\|}{C}}-C_{17}H_{35} + H_2O \rightarrow C_{34}H_{70} + CO_2 + H_2 \qquad (6)$$

In this reaction, hydrogen and carbon dioxide are formed. Also, some water is almost certainly produced because not all of the stearyl would be expected to condense to yield stearone. Large quantities of hydrogen, carbon dioxide and carbon monoxide have been shown to be produced in the pyrolysis of the lower acids(39,40). The reaction products contained ketones, paraffins and olefins. The carbon monoxide and olefins arise from secondary decomposition of ketones. The production of water and the evolution of carbon dioxide in the formation of ketones have been demonstrated (41,42).

Recent investigations to determine the origin of hydrocarbons and similar compounds in geologically significant areas has placed new emphasis on pyrolytic transformations of fatty acids. Model experiments have been set up which predict the distributions of hydrocarbons in sediments and petroleum deposits from fatty acids (43,44). In 1964, Jurg and Eisma (13) postulated a mechanism which accounts for the formation of both fatty acids and n-alkanes originating from fatty acids. The fatty acids and n-alkanes have carbon chains longer and shorter than that of the original acid. The scheme makes use of a radical reaction in which the initiation reaction is the decarboxylation of the fatty acid resulting in an alkyl radical. This will, in turn, react with the most abundant species present, which is the original fatty acid, producing a fatty acid radical and an n-alkane.

The fatty acid radical can split up into products which react with themselves and the other species to yield alkanes and fatty acids of a carbon number both greater and smaller than that of the original acid.

These mechanisms conform to the results obtained in the laboratory by heating behenic acid, $C_{21}H_{43}COOH$, which yielded n-alkanes of carbon number 7 to 34 in substantial amounts (13). The decarboxylation of the fatty acid is the essential step in obtaining carbon chains longer than the original acid. This was demonstrated by heating hexadecane under the same conditions as behenic acid, which did not yield any hydrocarbons whose chain length was greater than C-16. It must be pointed out that the reaction mechanisms for the pyrolysis of behenic acid are valid only for the conditions of the experiment using clay catalysts and an admixture

of water. In fact, under dry conditions, the tendency to isomerize was more pronounced as shown by high yields of branched paraffins which indicates the possibility that carbonium ions act as intermediates in this case.

IV. Formation of Petroleum by Pyrolytic-Type Transformations

A. Background

It is now almost universally accepted that petroleum was derived from the organic remains of plants and animals. The emphasis has now been placed on fatty acids, alcohols, free hydrocarbons, steroids, isoprenoids and amino acids. Fatty acids, however, have been given the most support (45).

The three major classes of substances that are involved in the life process are proteins, carbohydrates and lipids. The former two are labile and decomposed to substances that are not directly involved in petroleum formation, i.e., carbon dioxide, water, ammonia, nitrogen and hydrogen sulfide (46). The glycerides, on the other hand, are readily hydrolyzed, and the resulting higher fatty acids are relatively very resistant to bacterial destruction (47).

Sediments and crude oils have been found to contain the same type of hydrocarbons, and although the distribution of petroleum hydrocarbons differ from those in sediments, the close resemblance indicates that they were obtained from the same source: living matter (48). These sediments contain large amounts of paraffins as well as aromatic hydrocarbons, and the distribution of odd-number n-paraffins over even-numbered ones suggested

that these n-alkanes are formed from straight chain fatty acids (13). Fatty alcohols which are predominantly even-numbered in nature would have had to lose a hydroxyl group as well as a carbon atom. Nor could free hydrocarbons be the only source. Although the naturally occurring free hydrocarbons are odd-numbered, their paucity and the abundance of paraffins in sediments precludes the possibility that free hydrocarbons could account for them in any large measure.

Although a preference for odd-numbered n-paraffins is prevalent in recent sediments, an even distribution for odd and even numbered paraffins is found in analyses made on representative fractions of crude oil (49). In view of the fact that sediments represent intermediate stages in the formation of petroleum (50), the discrepancy in paraffin distribution presents an obvious difficulty. The accumulation of sedimentary organic material to form petroleum must have been done selectively, and explanations have been proposed as to how this was accomplished (48,51). In addition, Cooper (52) circumvents this difficulty by proposing that the decarboxylation of fatty acids produced reactive intermediates which subsequently react to give odd-numbered paraffins as well as fatty acids. The latter would repeat the process until eventually a secular equilibrium is attained in which there is no preference for odd or even numbered paraffins.

Most biological systems, on the other hand, have predominantly even-numbered carbon atoms (53), and even in instances where recent sediments have been found to contain odd and even-numbered fatty acids (52,54,55),

stearic and palmitic acids predominated, a circumstance which resembles biological systems.

B. Fatty Acids as Source Material

If fatty acids are to be considered the primary precursors of aliphatic hydrocarbons found in petroleum, only two pathways could bring about the transformation. Either the acids were acted upon by bacteria or other organisms having sufficient biochemical ability to effect the conversion, or the fatty acids were degraded by heat, time, catalytic action or other chemical considerations. Robinson (56) has suggested a third possibility in which hydrocarbons are synthesized in a manner analagous to Fischer-Tropsch processes, or by the action of radiation on methane according to the theory of Wilson (57,58). Although fatty acids, as well as other organic compounds, could furnish starting material for such processes, the evidence for the direct conversion of fatty acids, either biologically or chemically, is incontrovertible.

Hydrogenation of ketones, dehydration and reduction of double bonds are all common biochemical reactions (59). However, the reports of biological decarboxylation are few and irrelevant. Zobell (60) attributes the singular distribution of hydrocarbons in recent sediments to bacterial action although experiments under anaerobic conditions have not borne this out (61). Uranium catalyzes a bacterial decarboxylation reaction (62), but not very much petroleum could have been formed in this way. In plants, fatty acids have been found that contain one or more carbon than the corresponding paraffin (63,64), but the objections to this as a unique source have already been noted.

Because of the natural ubiquity and relative abundances of fatty acids, a likely explanation for petrogenesis has been found in pyrolytic-type transformations (65) and, indeed, evidence for such transformations occurring in sediments has been presented (66).

Among organic compounds fatty acids are considered to be the most stable. Extrapolation of the Arrhenius equation to ambient temperatures indicates that it would take many billions of years for them to decompose (67). However, at somewhat higher temperatures, naturally occurring catalysts and chemical interactions could shorten this time considerably.

V. Conditions

The earliest pyrolytic experiments were carried out in either glass or iron reaction vessels. Catalysts employed were various metals, metal oxides or metal chlorides. The nature of the product distribution was unique in each case. For example, 91% of the reaction products were stearone when stearic acid was destructively distilled in iron retorts (4). Zinc oxide, on the other hand, catalyzed hydrocarbon formation to the exclusion of ketone formation (68). In general, metals produce ketones, presumably due to the transitory formation of metal salts of the fatty acids which are readily decarboxylated. The use of water has been shown to produce paraffins that are straight chained (13).

In addition to metals, granular catalysts have often been used. Toschiichi Tokunaga (20) found that decarboxylation, dehydration and dehydrogenation were effected least easily when acid clay was used, while bauxite gave the best results. Brooks (47) has shown that silicate clays

are good catalyzers for the formation of paraffins and other hydrocarbons from fatty acids. Such clays occur naturally. High yields of olefins have been obtained by the thermal decomposition of saturated fatty acids at 300° using one or more of the following granular catalysts: activated acid clay, silica gel, alumina and bentonite (69). However, these same catalysts may also effect polymerization of the olefins once formed (47).

Because of inadequate investigations, the effects of pressure on fatty acid pyrolysis are essentially unknown. Kunkler (70) deduced from his work that while pressure may help to initiate a series of intermediate formation, it does not promote any further reaction. This was disputed by Engler (71) and Petrow (12), both of whom had produced naphthenes from fatty acid pyrolysis under pressure. Although pressures of up to 5,000 psi have been found in areas where fatty acids might be transformed to petroleum, Connant (72) has shown that pressure has nothing to do with the formation of petroleum.

Variations in heating time and temperature do influence pyrolytic reactions. Extremes in temperature like the use of catalysts can completely change the nature of the products. Optimum temperatures for the production of hydrocarbons seem to be 300-500°C with a duration of 2-5 hours, depending upon which catalyst is used. With bentonite clay, Jurg and Eisma were able to obtain paraffins at 200°C for 89 hours (13). The odd-predominance in the 27-37 carbon number range found in recent sediments which were not deeply buried has suggested that temperature has a great influence on the formation of n-alkanes from fatty acids,

because in more deeply buried sediments, this predominance tends to disappear (73).

VI. Other Biologically Important Acids

A. Phytanic Acid

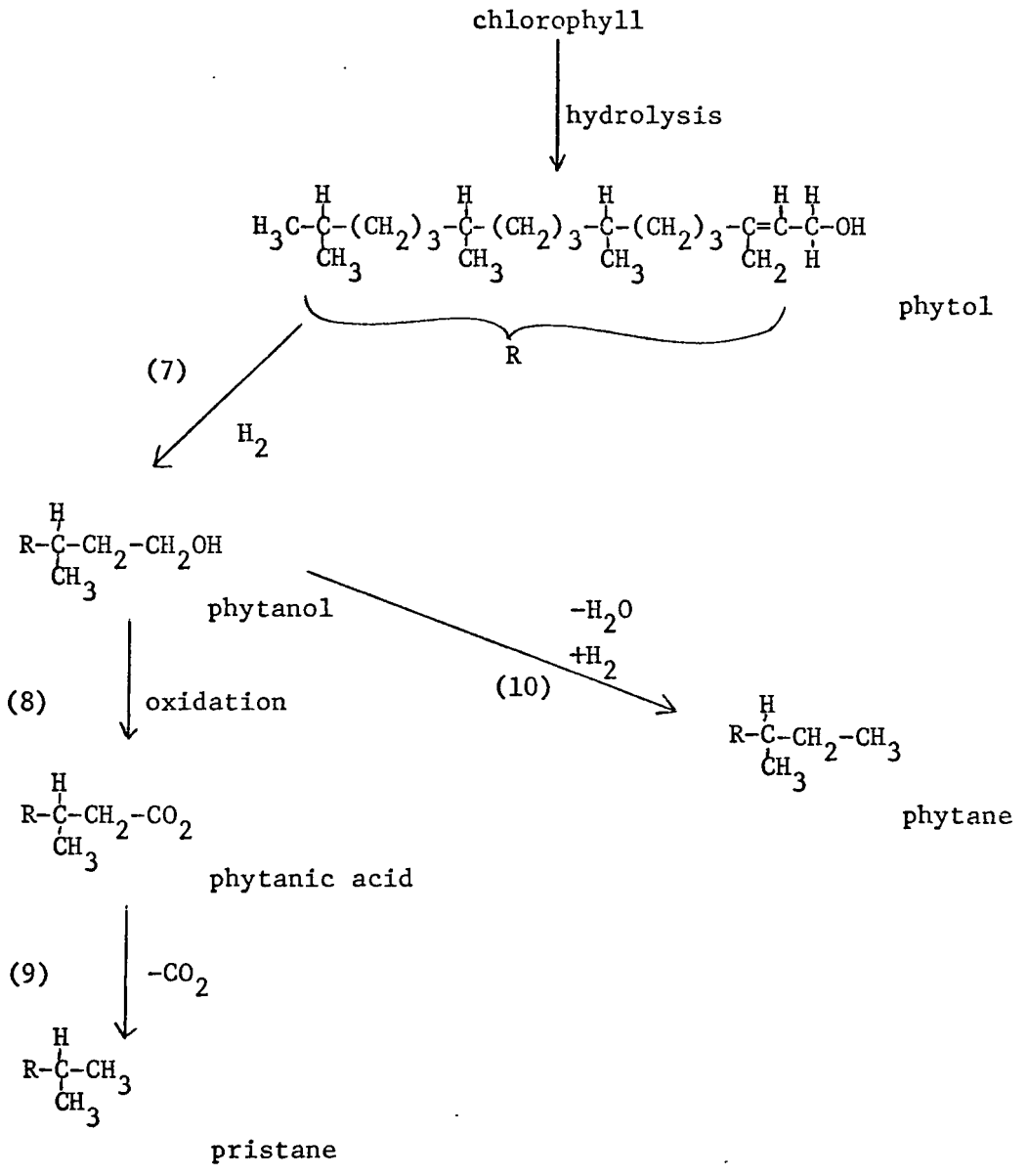
Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is obtained by selective reduction of the double bond, followed by oxidation of the alcohol group of phytol. Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) is found in nature and is obtained by hydrolysis from the porphyrin residue of chlorophyll to which it is attached by an ester linkage.

The discovery in 1939 by Treibs (74) of chlorophyl and heme derivatives in crude oil, whose side chains had been decarboxylated suggested that these aliphatic compounds would also be found in petroleum. Whitehead (75) discovered the occurrence of phytane, the corresponding paraffin, in petroleum and a C-19 isoparaffin which he suspected to be pristane, 2,3,10,14-tetramethylpentadecane. Decarboxylation of phytanic acid gives pristane.

Paraffins with methyl branches found in petroleum do not show preference for odd-carbon numbers, suggesting that their origin is from sources other than that of the n-paraffins. Their formation from isomerization of n-paraffins is conjectural (48). Meinschein (48) has cited isoprenoid substrates as being a possible source material for branched chain n-alkanes.

Of the isoprenoid-type hydrocarbons that have been specifically identified in petroleum, pristane was found to be the most abundant (76). Bendoraitis et al. (77) states that the likely choice of a precursor would be restricted to phytol, in view of the relative unreactivity of aliphatic side chains. Curphy (58) postulates a mechanism whereby the comparatively hydrophobic phytol forms a suspension in aqueous media, is subjected to epoxidation at the unsaturates site of the molecule, and is subsequently cleaved, yielding the ketone with two less carbon atoms. Later such ketones would become reduced as a result of certain geochemical phenomena, which are not specified, yielding the corresponding alkane. High temperatures would cause thermal cracking, the intermediates of which could result in innumerable geosynthetic derivatives. The objections to this are principally complexity of reactions involved from so obvious a direct precursor and the unaccountably favorable occurrence of pristane.

Bendoraites (76) found only two of the seven isoprenoid-type structures he identified in petroleum as true isoprenoids, i.e., having structures which are integral multiples of isoprene. While true isoprenoids are intermediates in biosynthetic pathways, the other five hydrocarbons must have resulted from degradative processes occurring in source beds. He further proposes mechanisms for the production of pristane and phytane from phytol and phytanic acid (see next page). Reactions 7, 8 and 10 have been carried out in the laboratory under various conditions (78,79), while decarboxylation of phytanic acid to pristane has only been proposed as a process occurring in the liver of Elasmobranch fishes (80).



Phytanic acid and norphytanic (pristanic) acid have been isolated from petroleum (81). Eglinton et al. (82) observed a predominance of these acids corresponding to a similar predominance of the corresponding branched n-alkanes in Green River Shale. This would seem to indicate that reaction (9) was the more important one.

B. Amino Acids

Erdman (50) has stated that for amino acids of proteins to be a source of hydrocarbons, they must be deposited in aquatic sediments in appreciable quantities. He based this statement on the findings of Zobell (83) who showed that bacteria could destroy amino acids so rapidly that the would not survive in the sediments. Abelson (84) and Marlett and Erdman (85) have found appreciable amounts of amino acids in recent sediments, indicating that they might be a source of smaller aliphatic hydrocarbons. In order for this to occur, two things must happen. The amino acids must be decarboxylated and they must undergo reductive deamination.

So far, only the decarboxylation of amino acids has been accomplished by pyrolysis. Abelson (86) has found this reaction to be first order, proportional to the concentration. He also estimated that alanine could persist for a billion years at room temperatures, but only a few hours at 250°C. Catalysts, light, and the presence of oxygen would make the lifetime of alanine exceedingly short.

No report of reductive deamination of alpha-amino acids has been published. Winter and Albro (87) have obtained characteristic amine profiles in pyrolysis at 300°C, and Valentyne (88) has obtained other amino acids in addition to the amines at somewhat lower temperatures. The low temperature pyrolysis of some substituted amino acids has yielded lactones, but this work is relevant to the production of hydrocarbons (89).

Statement of the Problem

While it is generally conceded that fatty acids from living organisms are a major contributor to the formation of hydrocarbons in petroleum, few attempts have been made to actually obtain such results in the laboratory. Earlier pyrolytic experiments were limited in the techniques of examination. The advent of gas-liquid chromatography, and the discovery of fatty acids and the corresponding odd-numbered paraffins, offered tantalizing possibilities for such experiments. One such experiment has been performed with behenic acid. Common saturated fatty acids in living organisms, however, are myristic, palmitic, and stearic.

Although phytol and phytanic acid have been postulated as precursors for branch-chain aliphatic hydrocarbons, no practical test of this hypothesis has been made. A proposed mechanism involving phytanic acid as an intermediate could be examined by pyrolytic decarboxylation of this compound.

Amino acids have been pyrolyzed. However, the only hydrocarbons that were of interest were those of comparatively low molecular weight. Consequently, in the pyrolyses that were performed, possible production of higher molecular weight hydrocarbons was either not examined or not reported.

It is the purpose of this work to pyrolyze the compounds in question to see if hydrocarbons are produced. An examination will be made for paraffins of higher carbon number than the pyrolyzed acid. Certain experiments will be performed to obtain results under different conditions. Paraffin distributions for a particular fatty acid will be determined.

Phytanic acid will be pyrolyzed to see if the decarboxylation of this acid is a route to the production of branched alkanes, particularly pristane.

An examination of the pyrograms of amino acids will be made to determine if hydrocarbons are produced.

II.

EXPERIMENTAL

EXPERIMENTAL

Pyrolysis and Extraction Equipment and Techniques

With the exception of phytanic acid, all fatty acids were pyrolyzed according to the procedure described here. The reaction mixture (acid, clay, and water) was inserted in the appropriate reaction tube purged with hydrogen (except in the case of the palmitic acid pyrolysis in air), and heated in a muffle furnace previously set for 300°C. Reaction tubes for all but the pyrolysis of palmitic acid in air were 1" x 5" stainless steel cylindrical tubes fitted with a Swagelok cap. With the cap properly fitted, the tubes were checked with a helium leak detector and found to be incompletely sealed. Subsequent pyrolyses in these tubes showed no charring of the contents. This system was used for most of the pyrolyses. A completely sealed system was obtained by the use of a molybdenum lubricating compound, which reduced friction in the threads of the cap and permitted a tighter seal. The pyrolysis of palmitic acid in air was done in a 1 1/2" x 6 1/2" stainless steel cylindrical tube with a screw cap.

After cooling, the extractable material from the residue was transferred to a small beaker with 25 ml of a benzene and methanol mixture (in the ration of 3:1, respectively). This mixture was evaporated to dryness under a stream of nitrogen at 40°C. The remaining residue was extracted with 9 ml of heptane and then was transferred to a heptane-washed activated silica gel column. The aliphatic hydrocarbons were eluted with 15 ml of heptane. In some cases, the residue was further

extracted with benzene and methanol. These portions were saved for future analyses.

Materials

The fatty acids were taken from stock bottles sold by Eastman Organic Chemicals. Prior to pyrolysis, an amount comparable to the amount to be pyrolyzed was treated and analyzed exactly as the residue after pyrolysis is treated. A blank was also run on the bentonite clay (Magcobar-magcogel, Wyoming Bentonite). Figure 10C is typical of the blank runs.

The amino acids and the phytol used in this experiment were obtained from Nutritional Biochemical Corporation, Cleveland. Silica gel (J. T. Baker Chemical Company) was activated at 450°C for 24 hours prior to use.

The Bacillus cereus cells were grown and extracted in the laboratory (90). The methanol fraction represents the lipid contents of 7.7 g of dried Bacillus cereus and weighed 0.12 grams. This was mixed with bentonite clay, pyrolyzed, and treated in a manner similar to the procedure for fatty acids.

Preparation of Phytanic Acid

Ten grams of phytol were dissolved in 500 ml of ethanol and 1 gram of 10% palladium on charcoal was added. The phytol was hydrogenated for twelve hours at room temperature under 25 psi of hydrogen pressure. The resulting mixture containing phytane, pristane and dihydrophytol was oxidized according to the method of Karrer and Epprecht (91) and distilled

in vacuo. The fraction boiling from 140-170°C at 0.7 mm Hg was collected, re-oxidized, and redistilled. The fractions boiling off between 180-195°C (0.7 mm Hg) and 170-175°C in the second distillation (0.3 mm Hg) were combined.

The impure phytanic acid (5 ml) was dissolved in methanol and 10 ml of concentrated sulfuric acid. The mixture was refluxed at 100°C (bath temperature). After 8 hours the lighter material was stripped off under vacuum at 60° and the impure methyl phytanate was vacuum distilled. Selected fractions of the distillate were examined by gas chromatography and, by recombining fractions rich in methyl phytanate and redistilling, a products was obtained boiling at 180°C (0.5 mm Hg) which was 99% pure methyl phytanate.

The methyl phytanate was saponified, washed with ether, neutralized with 0.1 M HCl, and extracted with ether. Total yield of phytanic acid for the procedure was about 20%.

Pyrolysis Attachment to Gas Chromatograph

A diagram of the pyrolysis chamber used for pyrolyzing the phytanic and the amino acids is shown in Figure 1. It is placed outside of the gas chromatographic oven and the inlet side of the column attaches directly to the pyrolysis chamber. Helium at 15 psi is forced through .03 I.D. capillary tubing and with all valves closed is sent directly through the column. When the two gas valves which control the outlet and inlet sides of the pyrolysis chamber are opened, gas is flushed through the pyrolysis chamber. Both valves are equipped with pressure gauges and

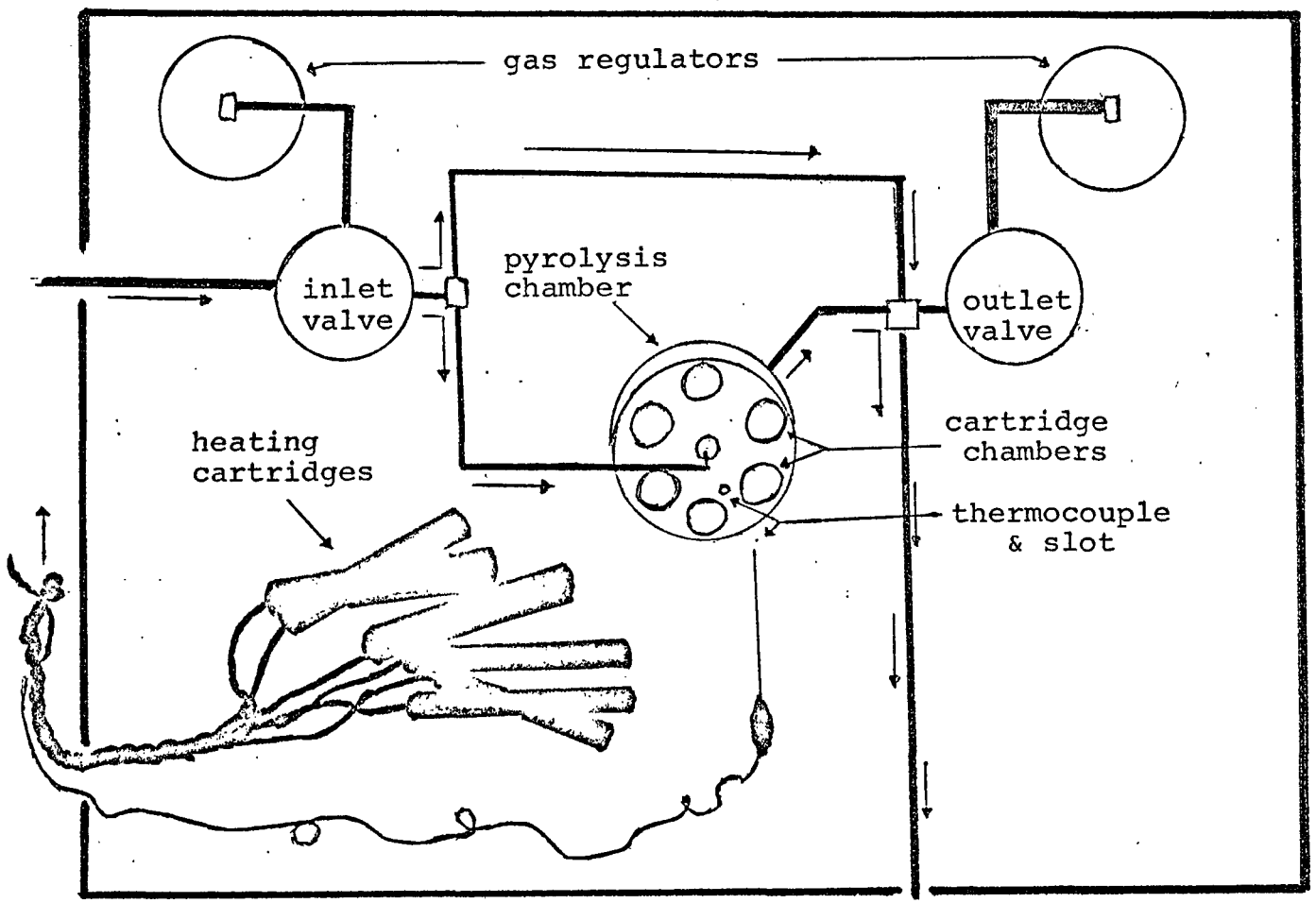


FIGURE 1. DIAGRAM FOR PYROLYSIS CHAMBER

regulators so that the inlet and outlet pressures may be controlled. Heating tape is wrapped around all tubing from the pyrolysis chamber to the oven and, during injection, the temperature of this tubing is maintained at least as high as the temperature of the pyrolysis chamber.

A typical pyrolysis run would best describe the operation. Aqueous solutions of the amino acids are placed in a glass liner (1/4" O.D., 1/32" I.D. and 5" long) and the water is evaporated in an oven at 100°C for 5 minutes. The liner is inserted in a nickel-steel jacket which, in turn, is inserted in the gas chamber. Teflon washers are placed in nuts which are screwed to the jacket. These washers are in contact with the glass liner so that the contents are not in contact with metal surfaces inside the pyrolysis chamber. Tubing (1/16" O.D.) leading directly to the chromatographic column is attached to the metal jacket and the system is flushed with helium by opening the outlet valve. Tubing from the helium source is then attached and the outlet valve is closed. In the case of phytanic acid, the glass liner is not used. Heating cartridges and a temperature sensor from an Alnor thermostatic heating unit are inserted in the pyrolysis chamber, and the desired temperature is achieved within 2 minutes.

Prior to injection, the temperature is brought to 300°C (comparable to most injection systems), and after equilibration, the contents of the chamber are injected into the column by flushing the system for ten seconds. The system is cooled and a standard is inserted, brought to injection temperature and injected in the same manner. Usually there is enough residual material remaining from the pyrolysis that this becomes

an internal standard. The system is then cleaned and a blank is run prior to subsequent analysis.

Gas-Liquid Chromatography

Table III gives the systems which are referred to in the Figures with reference to the conditions of an analysis. Samples were injected with a Hamilton microliter syringe. No split was used so that all of an injected sample was separated and analyzed. Peak identification was accomplished by use of internal standards, by external standards and different columns, or by the aid of mass spectrometric analysis.

TABLE III

ANALYSIS SYSTEMS

System	Instrument	Conditions
A	Barber Colman Series 5000 with Hamilton Injector and Flame Detector	Injector: 295°C Detector: 300°C Flame: H_2 at 10 psi Air at 30 psi
B	Same as (A), except with pyrolysis unit attached	Same as (A) except no Injector used
C	F & M Model 810 equipped with Flame Ionization Detector	Injector: 300°C Detector: 290°C Flame: H_2 at 18 psi Air at 30 psi

Stainless Steel Columns*

Dimensions	Phase	Carrier Gas Pressure
1. .03 cm x 300'	Polysev [m-bis-m(phenoxy-phenoxy)-phenoxy benzene]	15 psi
2. .03 cm x 500'	SF-96 (silicone fluid)	15 psi
3. .03 cm x 650'	OV-17	20 psi
4. .02 cm x 500'	Igepal (nonyl phenoxy polyoxyethylene ethynol)	20 psi

*Handy and Harmon Tube Co., Inc., Norristown, Pennsylvania

III.

RESULTS

RESULTS

Pyrolysis of Palmitic Acid in Air

A 1.0 gram sample of palmitic acid was mixed with four grams of bentonite clay and 2.5 grams of water. The mixture was placed in a screw-capped reaction tube and without purging was pyrolyzed for two hours at 300°C. The tube contained small openings which allowed volatile substances to escape. The residue, which was black, was extracted and fractionated according to the procedures described in the Experimental section. Figure 2 shows the gas chromatographic analysis of the heptane and benzene fractions, respectively.

Pyrolysis of Palmitic Acid in a Sealed System

The reaction mixture consisted of a 1 gram sample of palmitic acid mixed with 4 grams of bentonite and 2.5 grams of water. The sample tube (Swagelok type) was purged with hydrogen prior to capping. Since these type tubes were not completely vacuum tight (see Experimental), the tube was sealed with a molybdenum sealing compound. Prior analysis showed this compound to contain no extractable organic material. The sample was heated to 300°C for two hours and analyzed (Figure 3). No attempt was made to measure the pressure in the system. Upon opening the tube after cooling, a sharp report of rapidly expanding gas was produced, indicative of gaseous compounds being formed during the reaction. The contents of the tube were odiferous and the residue was gray.

FIGURE 2

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF PALMITIC ACID PYROLYSIS PRODUCTS

Heptane Fraction:	Benzene Fraction:
1 µl injected out of 30 µl of solution	System A
System A	Column: OV-17
Column: SF-96	Temperature: 255°C
Temperature: 200°C	Recorder Span: X 5
Recorder Span: X 5	Attenuation: 30
Attenuation: 30	

Identification*

1. C_{11}	10. C_{17}
2. C_{12}	11,12,13. Δ-C_{18}
3. C_{13}	14. C_{18}
4. C_{14}	15. C_{19}
5,6,7. Δ-C_{15}	16. C_{19}
8. C_{15}	17. unidentified
9. C_{16}	18. position where C_{20} would be

*tentative

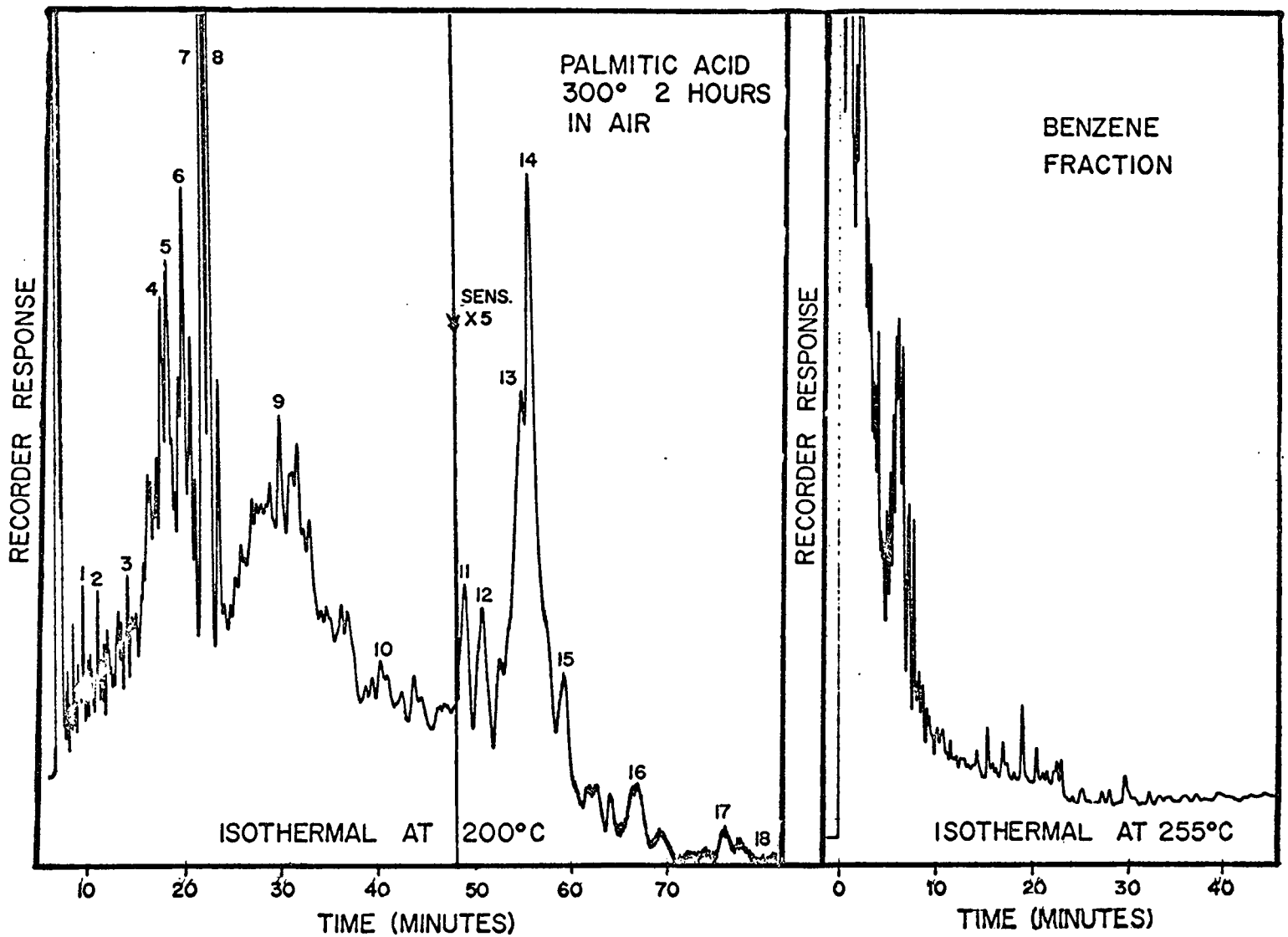

PALMITIC ACID
300° 2 HOURS
IN AIR
SENS.
X5
RECORDER RESPONSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
ISOTHERMAL AT 200°C
10
20
30
40
50
60
70
TIME (MINUTES)
BENZENE
FRACTION
RECORDER RESPONSE
ISOTHERMAL AT 255°C
0
10
20
30
40
TIME (MINUTES)

FIGURE 3

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF PALMITIC ACID PYROLYSIS PRODUCTS (SEALED SYSTEM)

1 µl injected out of 4.5 ml sample

System: A

Column: Polysev

Temperature: 100°C, programmed at 2°/minute to 200°C

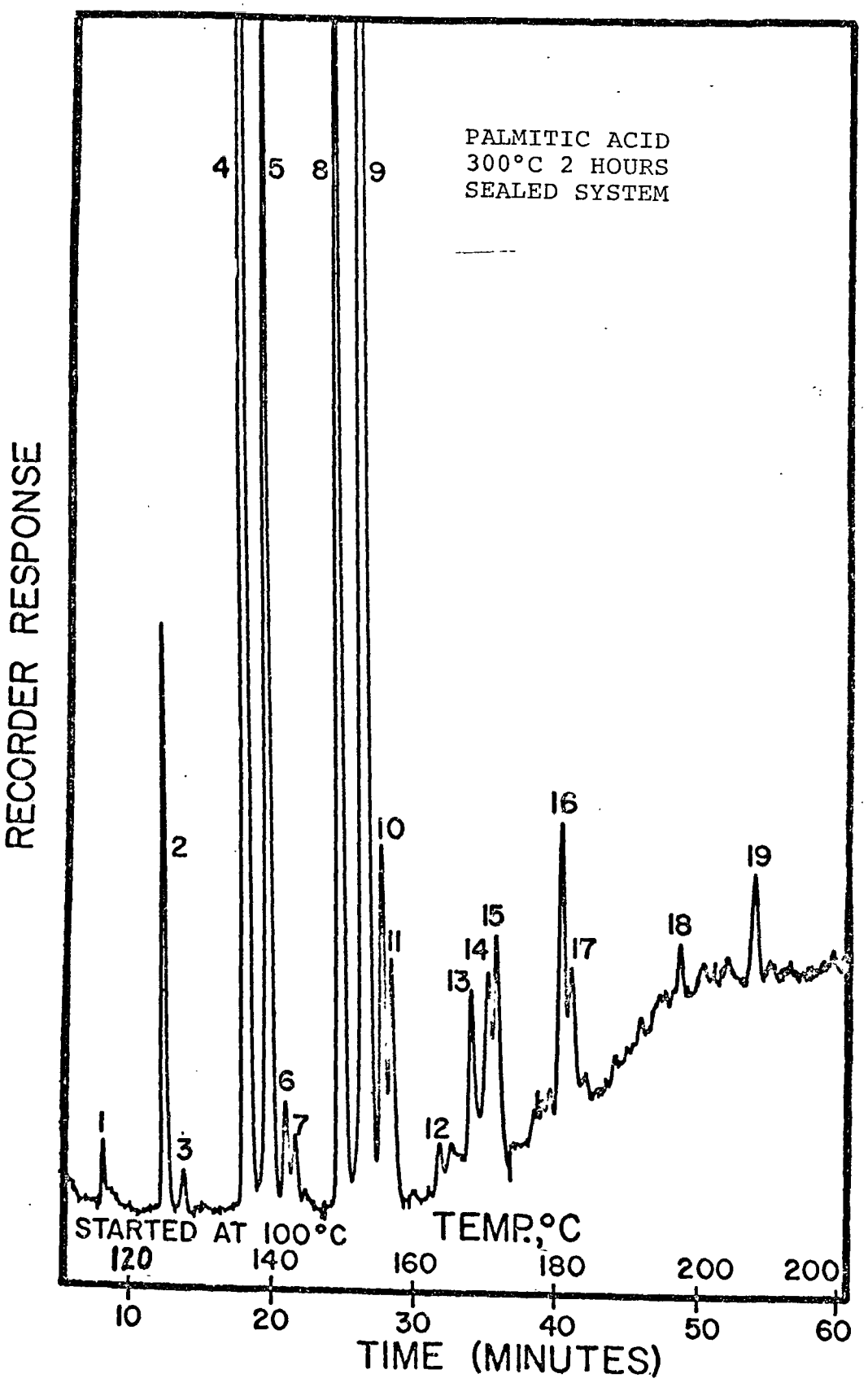
Recorder Span: X 1

Attenuation: 30

Identification:*

1. C_{12}
2. C_{13}
3. Δ-C_{13}
4. C_{14}
5,6,7. Δ-C_{14}
8. C_{15}
9,10,11. Δ-C_{15}
12. C_{16}
13. Δ-C_{16}
14,15. Δ-C_{16}
16. C_{17}
17. Δ-C_{17}
18. C_{18}
19. C_{19}

*tentative


PALMITIC ACID
300°C 2 HOURS
SEALED SYSTEM
RECORDER RESPONSE
STARTED AT 100°C
TEMP.,°C
120
140
160
180
200
200
10
20
30
40
50
60
TIME (MINUTES)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

Pyrolysis of Palmitic Acid Unsealed

The same reaction mixture as in previous experiments was used. The tube was purged with hydrogen and a Swagelok cap tightened over the top. No sealing compound was used. The sample was pyrolyzed as in the preceding experiments. After cooling, the system was opened and analyzed (Figure 4). The residue was gray and the products were odiferous.

There appeared to be hydrocarbons of higher molecular weight in the sealed pyrolysis and pyrolysis in air than in the unsealed, hydrogen purged system. The experiment was repeated with the same results.

Myristic Acid

Because of the inconclusive results with palmitic acid, myristic acid was pyrolyzed in a sealed and an unsealed system. One gram of myristic acid, 3 grams of bentonite, and 2.5 grams of water were used in each case. The system was purged with hydrogen prior to heating at 300°C for 2 hours. The results are shown in Figures 5 and 6. Since the unsealed system appears to give a wider distribution of products as well as some higher molecular weight material in both the benzene and heptane fractions, it was decided to use this system in subsequent experiments.

Stearic Acid

An 0.5 gram sample of stearic acid was mixed with bentonite (3 grams) and 0.5 grams of water. The tube was purged with hydrogen, closed and

FIGURE 4

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF PALMITIC ACID PYROLYSIS PRODUCTS (UNSEALED SYSTEM)

1 µl out of 20 µl injected

System: A

Column: Polysev

Temperature: 100°C, programmed at 2°/minute to 200°C

Span: X 5

Attenuation: 10

Identification: *

1. C_{12}
2. C_{13}
3. Δ-C_{13}
4. C_{14}
5,6. Δ-C_{14}
7,8. unidentified
9. C_{15}
10,11. Δ-C_{15}
12,13. unidentified
14. C_{17}
15. Δ-C_{17}

*tentative

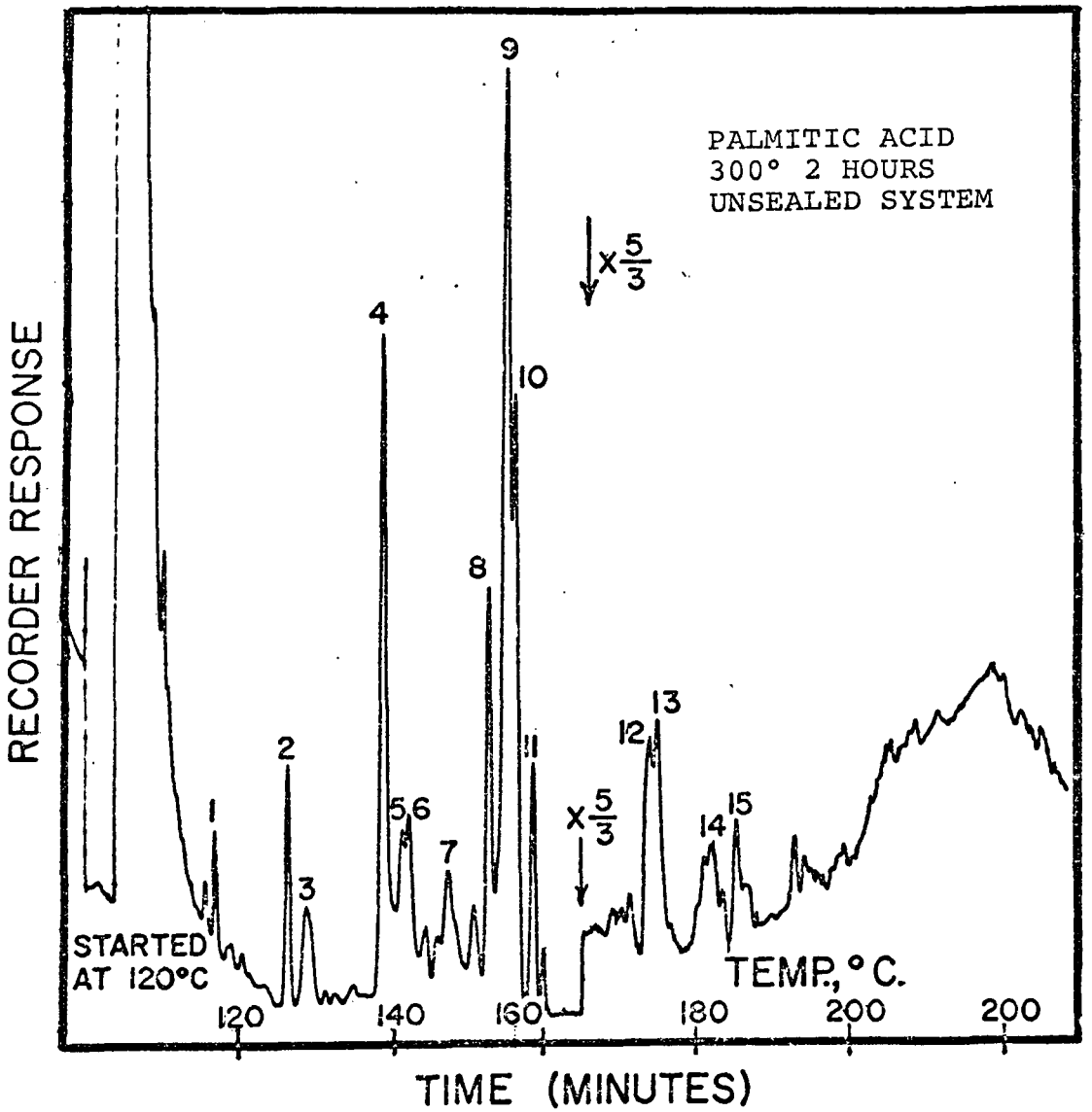

PALMITIC ACID
300° 2 HOURS
UNSEALED SYSTEM
RECORDER RESPONSE
STARTED AT 120°C
TEMP., °C.
TIME (MINUTES)
120
140
160
180
200
200
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
X 5/3
X 5/3

FIGURE 5

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF MYRISTIC ACID PYROLYSIS PRODUCTS (SEALED SYSTEM)

1 µl injected out of 20 µl

System: A

Column: SF-96

Temperature: 200°C

Recorder Span: X 1

Attenuation: 30

Identification:

1. C_{12}
2. Δ-C_{13}
3. C_{13}
4. C_{14}
5. C_{15}
6. C_{16}
7. C_{17}
8. C_{18}

*tentative

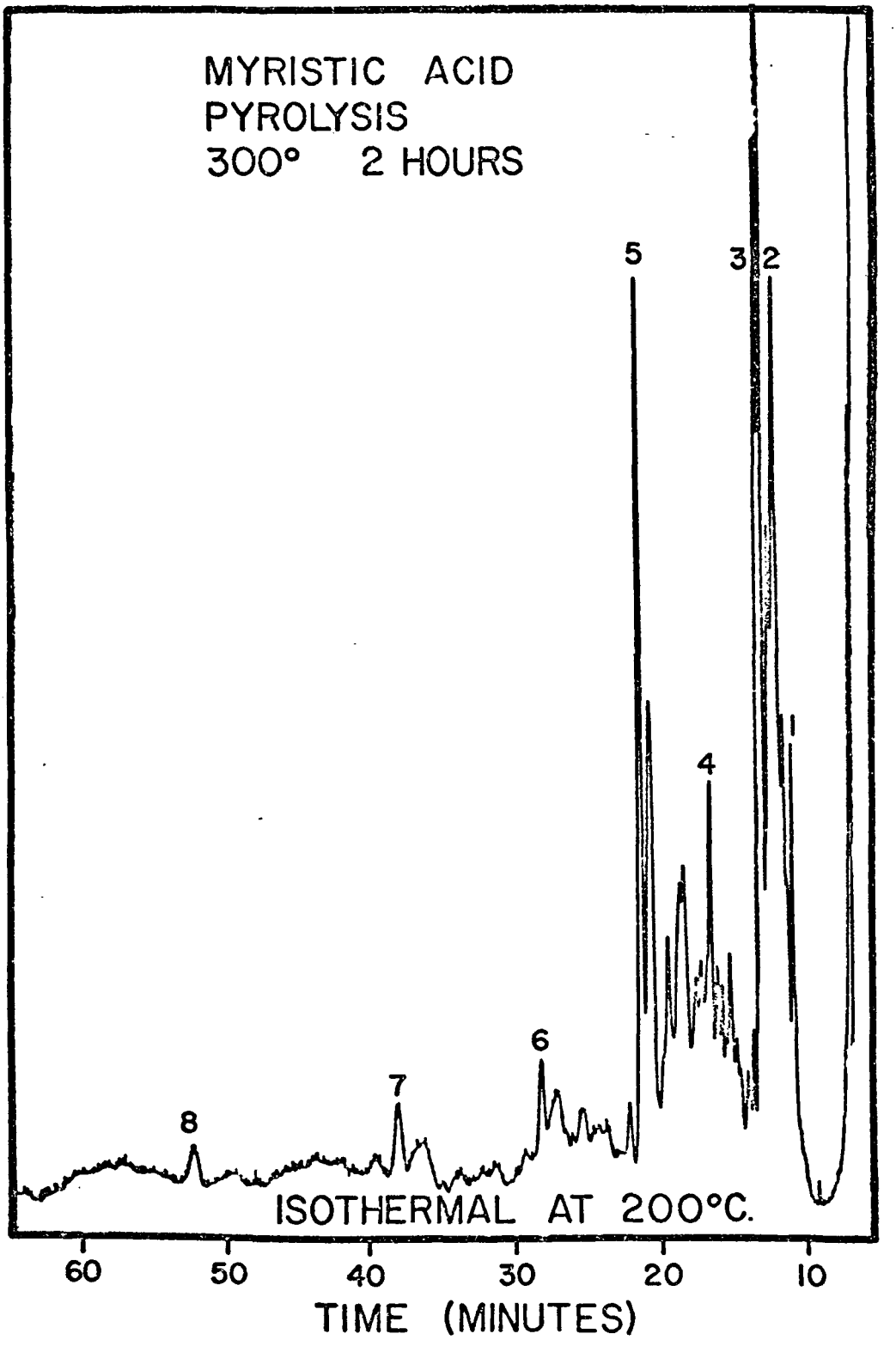

MYRISTIC ACID
PYROLYSIS
300° 2 HOURS
5
3
2
4
6
7
8
ISOTHERMAL AT 200°C.
60
50
40
30
20
10
TIME (MINUTES)
RECORDER RESPONSE

FIGURE 6

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF MYRISTIC ACID PYROLYSIS PRODUCTS (UNSEALED SYSTEM)

1 µl injected out of 25 µl

System: A

Column: SF-96

Temperature: 200°C

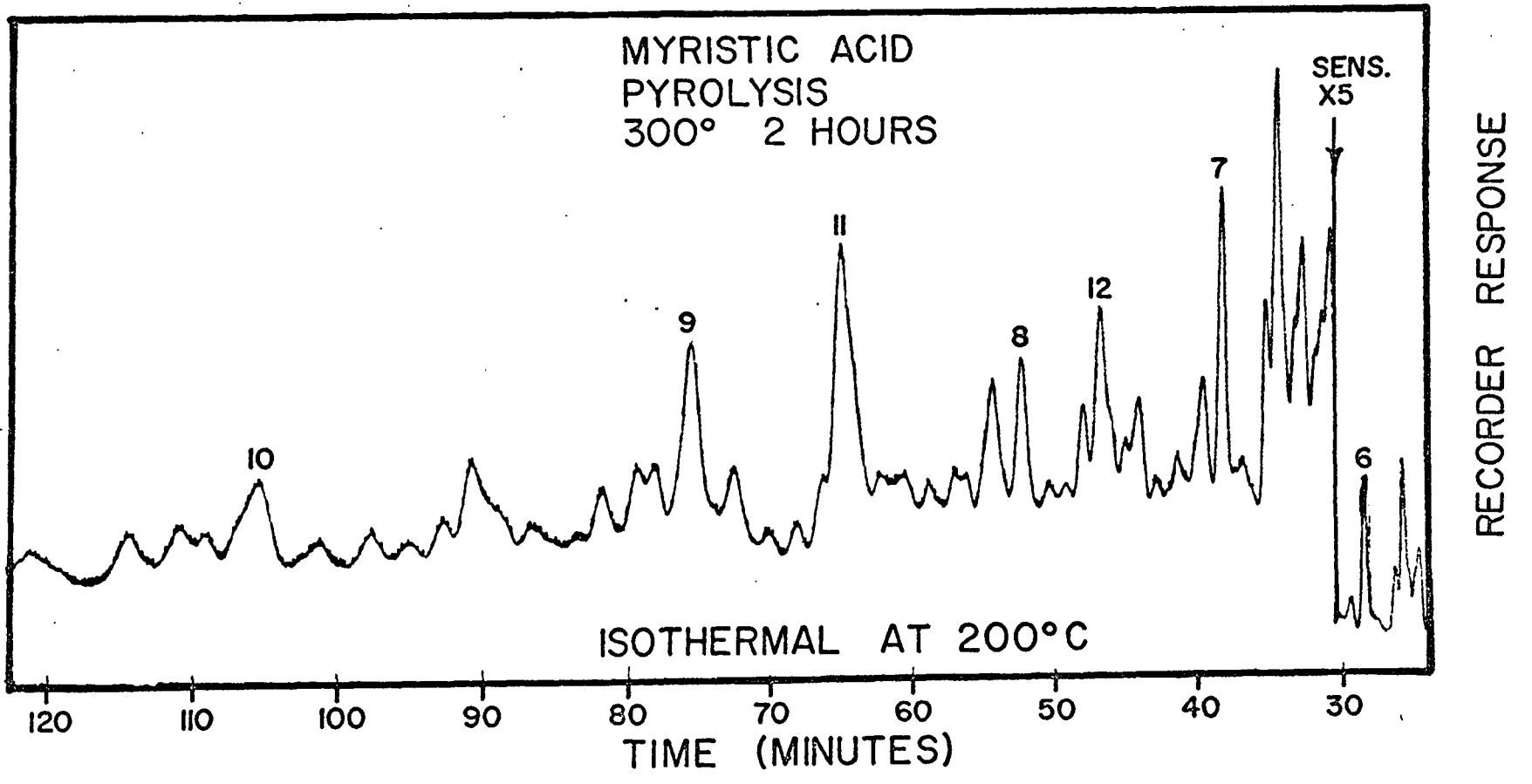
Recorder Span: X 1

Attenuation: 30

Identification:*

6. C_{16}
7. C_{17}
8. C_{18}
9. C_{19}
10. C_{20}
11. unidentified
12. unidentified

*tentative


MYRISTIC ACID
PYROLYSIS
300° 2 HOURS
SENS.
X5
10
9
11
8
12
7
6
ISOTHERMAL AT 200°C
120
110
100
90
80
70
60
50
40
30
TIME (MINUTES)
RECORDER RESPONSE

pyrolyzed for 2 hours at 300°C. Analysis of the heptane fraction is shown in Figure 7.

Mixed Fatty Acids

Two acids, stearic and palmitic, were used in this experiment. They were mixed with one gram of water and 3 grams of bentonite and pyrolyzed as described for stearic acid above. The same amounts of acids were then pyrolyzed at 300°C for four hours to see what effect a longer heating time would have on the distribution of products. Results are shown in Figures 8 and 9.

Pyrolysis of Methanol Fractions of *Bacillus cereus*

The lipid fraction of *B. cereus* was obtained by methanol extraction as described in the Experimental section. A 0.12 gram sample was mixed with 1.5 grams of bentonite and 0.15 grams of water and pyrolyzed. All procedures were the same except that the residue was extracted three times with 5 ml of pentane and passed through a silica gel column. The sample was evaporated to about 100 µl. The results are shown in Figure 10A. Figure 10B is a pyrolysis of 1.1 grams of whole cells. The amount of hydrocarbons present is approximately proportional to the lipid material present, i.e., about 1/7 of the hydrocarbons produced in the pyrolysis of the methanol extract. Figure 10C is a blank run of the bentonite clay used in the pyrolysis experiments.

FIGURE 7

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF STEARIC ACID PYROLYSIS PRODUCTS

2 µl injected out of 20 µl

System: C

Column: Igepal CO 990

Temperature: 125°C for 10 minutes, programmed at 6°/minute to 200°C

Range: 100

Attenuation: 1

Identification:*

1.	C_{14}	10.	C_{18}
2.	Δ-C_{14}	11.	C-Δ-C_{18}
3.	C_{15}	12.	C_{19}
4.	Δ-C_{15}	13.	C_{20}
5.	C_{16}	14.	C_{21}
6,7.	Δ-C_{16}	15.	C_{22}
8.	C_{17}	16.	C_{23}
9.	Δ-C_{17}	17.	C_{24}

*tentative

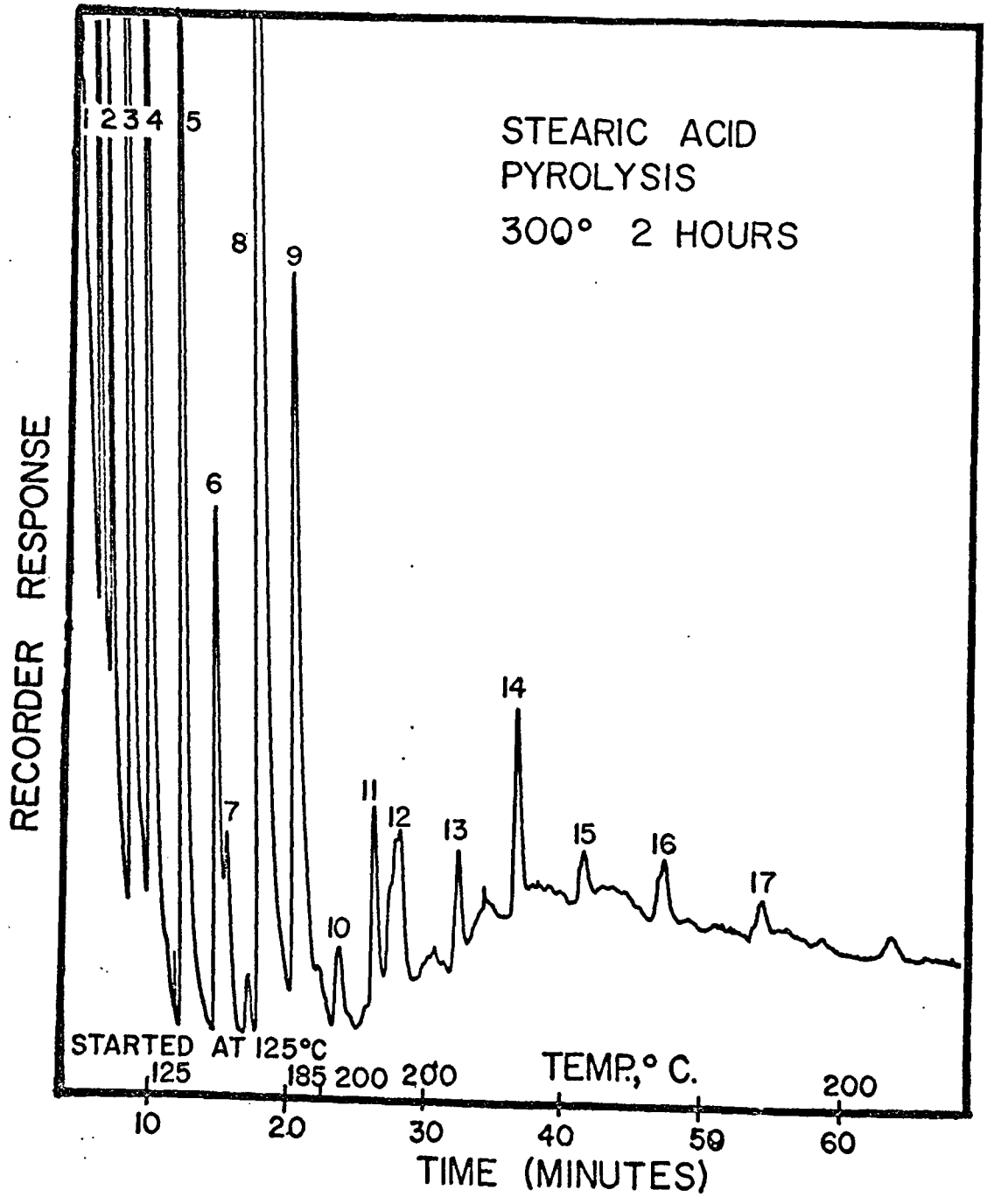

STEARIC ACID
PYROLYSIS
300° 2 HOURS
RECORDER RESPONSE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
STARTED AT 125°C
125 185 200 200 TEMP., ° C. 200
10 20 30 40 50 60
TIME (MINUTES)

FIGURE 8

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF MIXED FATTY ACID PYROLYSIS PRODUCTS AT 300°C FOR TWO HOURS

2 µl injected out of 4.5 µl

System: C.

Column: Igepal CO 990

Temperature: 300°C for 10 minutes, programmed at 6°C/minute to 200°C

Range: 100

Attenuation: 1

Identification:*

1.	C_{14}	6.	C_{19}
2.	C_{15}	7.	C_{20}
3.	C_{16}	8.	C_{21}
4.	C_{17}	9.	C_{22}
5.	C_{18}	10.	C_{24}

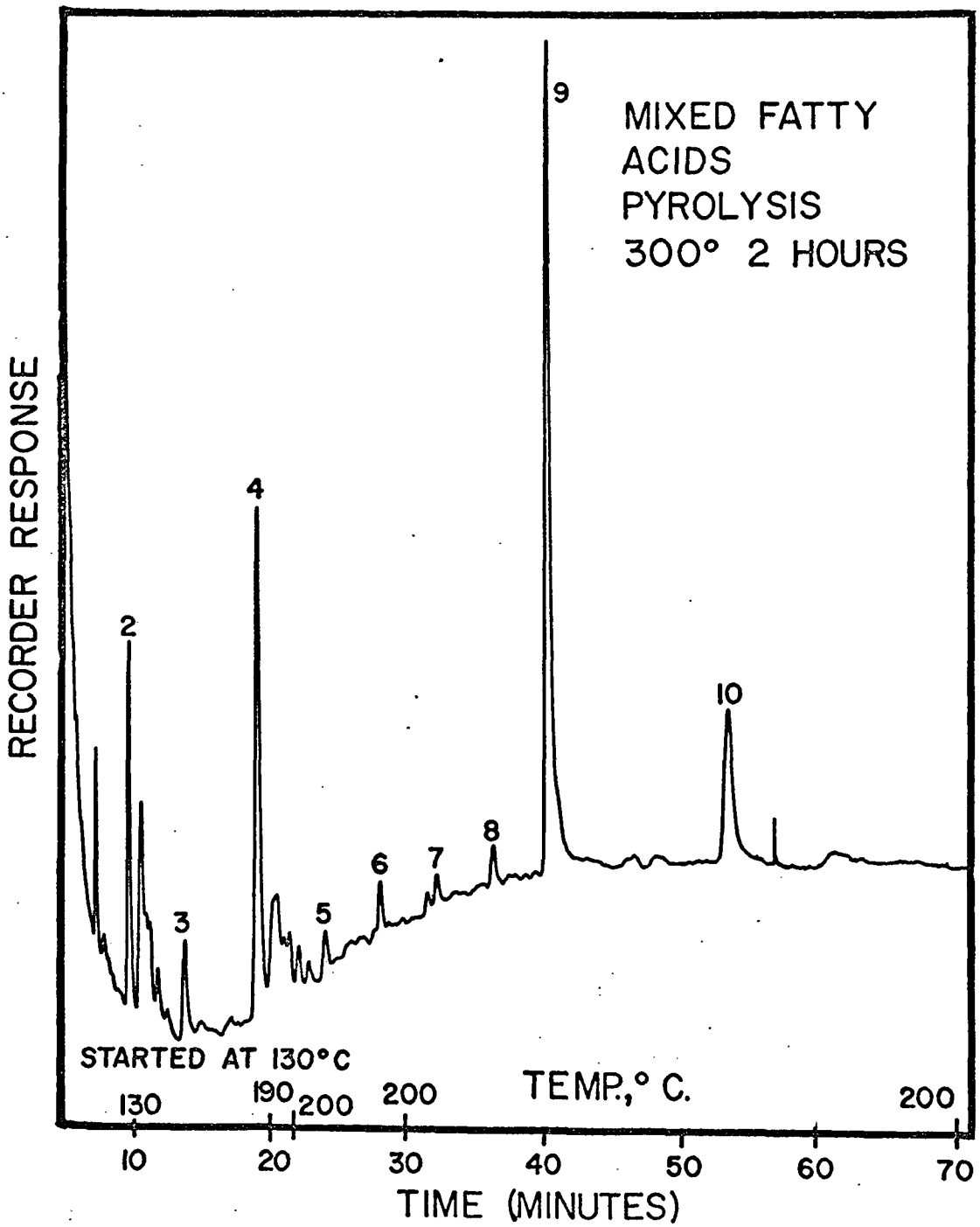

MIXED FATTY
ACIDS
PYROLYSIS
300° 2 HOURS
RECORDER RESPONSE
9
4
2
10
8
7
6
3
5
STARTED AT 130°C
130
190
200
200
TEMP.,° C.
200
10
20
30
40
50
60
70
TIME (MINUTES)

FIGURE 9

GAS CHROMATOGRAPHIC ANALYSIS OF THE HEPTANE FRACTION OF MIXED FATTY ACID PYROLYSIS PRODUCTS AT 300°C FOR FOUR HOURS

2 µl injected out of 4.5 µl

System: C

Column: Igepal CO 990

Temperature: 100°C for 10 minutes, programmed at 6°/minute to 200°C

Range: 10

Attenuation: 4

Identification:*

1. C_{13}
2. C_{14}
3. Δ-C_{14}
4. C_{15}
5. Δ-C_{15}
6. C_{16}
7. Δ-C_{16}
8. C_{17}
9. Δ-C_{17}
10,11. Δ-C_{18}
12. C_{18}
13. C_{20}
14. C_{21}

*tentative

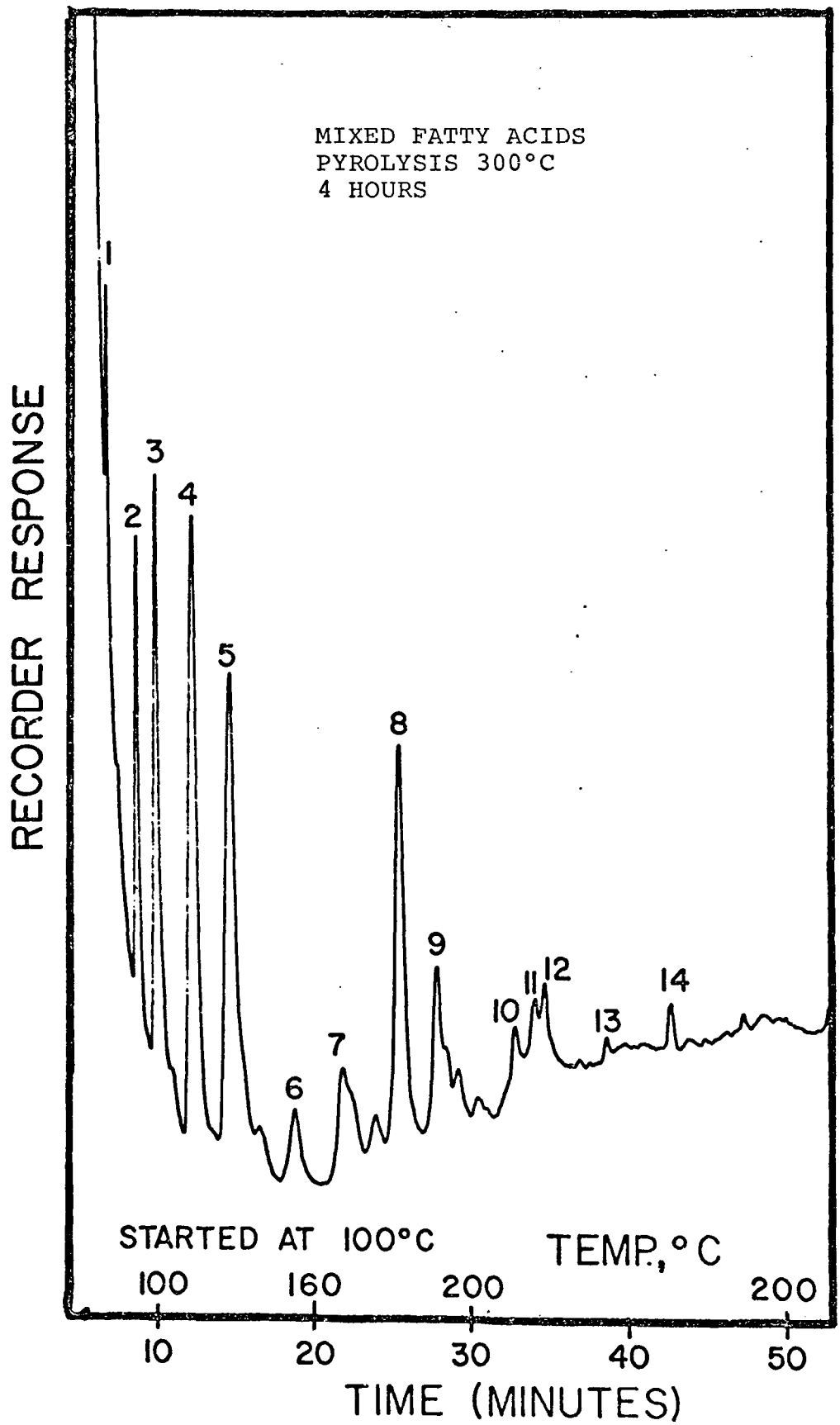

MIXED FATTY ACIDS
PYROLYSIS 300°C
4 HOURS
RECORDER RESPONSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
STARTED AT 100°C
TEMP., °C
100
160
200
200
10
20
30
40
50
TIME (MINUTES)

FIGURE 10.

GAS CHROMATOGRAPHIC ANALYSIS OF THE PENTANE FRACTION OF

A. PYROLYSIS PRODUCTS OF METHANOL FRACTION OF BACILLUS CEREUS

B. PYROLYZED WHOLE CELLS OF BACILLUS CEREUS

C. PYROLYZED BENTONITE CLAY

1 µl injected out of 100 µl

System: C

Column: Igepal CO 990

Temperature: 130°C for 10 minutes, programmed at 6°/minute to 200°C

Range: 100

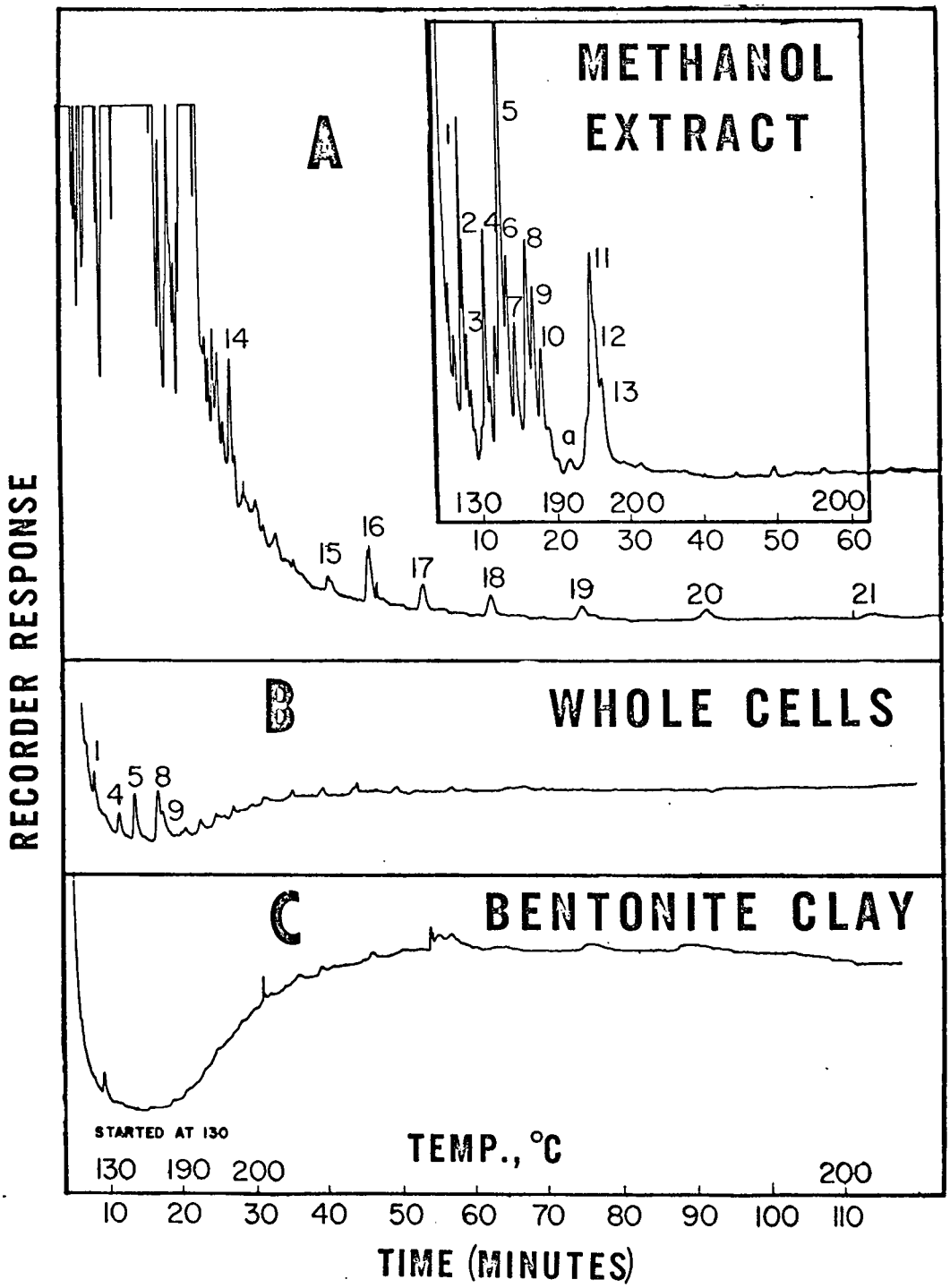
Attenuation: 1

Identification: *

1,2,3. Δ-C_{14}	14. C_{18}
4. C_{15}	15. C_{21}
5,6,7. Δ-C_{15}	16. C_{22}
8. C_{16}	17. C_{23}
9,10. Δ-C_{16}	18. C_{24}
a. Pristane	19. C_{25}
11,12,13. Δ-C_{17}	20. C_{26}

*tentative

BACILLUS CEREUS PYROLYSIS 300° 2 HOURS



Pyrolysis of Phytanic Acid

A previous pyrolysis of 100 mg of phytanic acid failed to show any hydrocarbons in the heptane fraction. It was not known whether this result was due to the low temperature and heating time (300°C, 2 hours) or the smaller quantity as compared to the previous fatty acid runs. Therefore, phytanic acid was pyrolyzed in a specially-built pyrolysis chamber (see Experimental) which attaches to an inlet side of a capillary gas chromatographic column.

The sample consisted of 3 µl of phytanic acid (a viscous liquid at room temperature) and 1 µl of water. The system was purged with helium and completely sealed. As with previous pyrolyses in closed systems, no attempt was made to measure the pressure developed during heating. The sample was pyrolyzed at 500°C for 1/2 hour. The analysis is shown in Figure 11.

The inset of Figure 11 is the initial injection and the larger chromatogram is a second 10-second injection of the same pyrolyzed sample. Only the portion of both chromatograms showing the higher molecular weight components were found to be similar with respect to peak size. Testing the system with a known standard has shown that a much larger proportion of the lighter material is flushed from the system in the first injection.

Amino Acid Pyrolysis

The pyrolyses of the amino acids were also carried out in the pyrolysis unit which attaches to the gas chromatograph. In this case, the glass liner which inserts into the heating chamber was used.

FIGURE 11

GAS CHROMATOGRAPHIC ANALYSIS OF THE PYROLYSIS PRODUCTS OF PHYTANIC ACID

Injection: 10-second flush

System: B

Column: SF-96

Temperature: 200°C

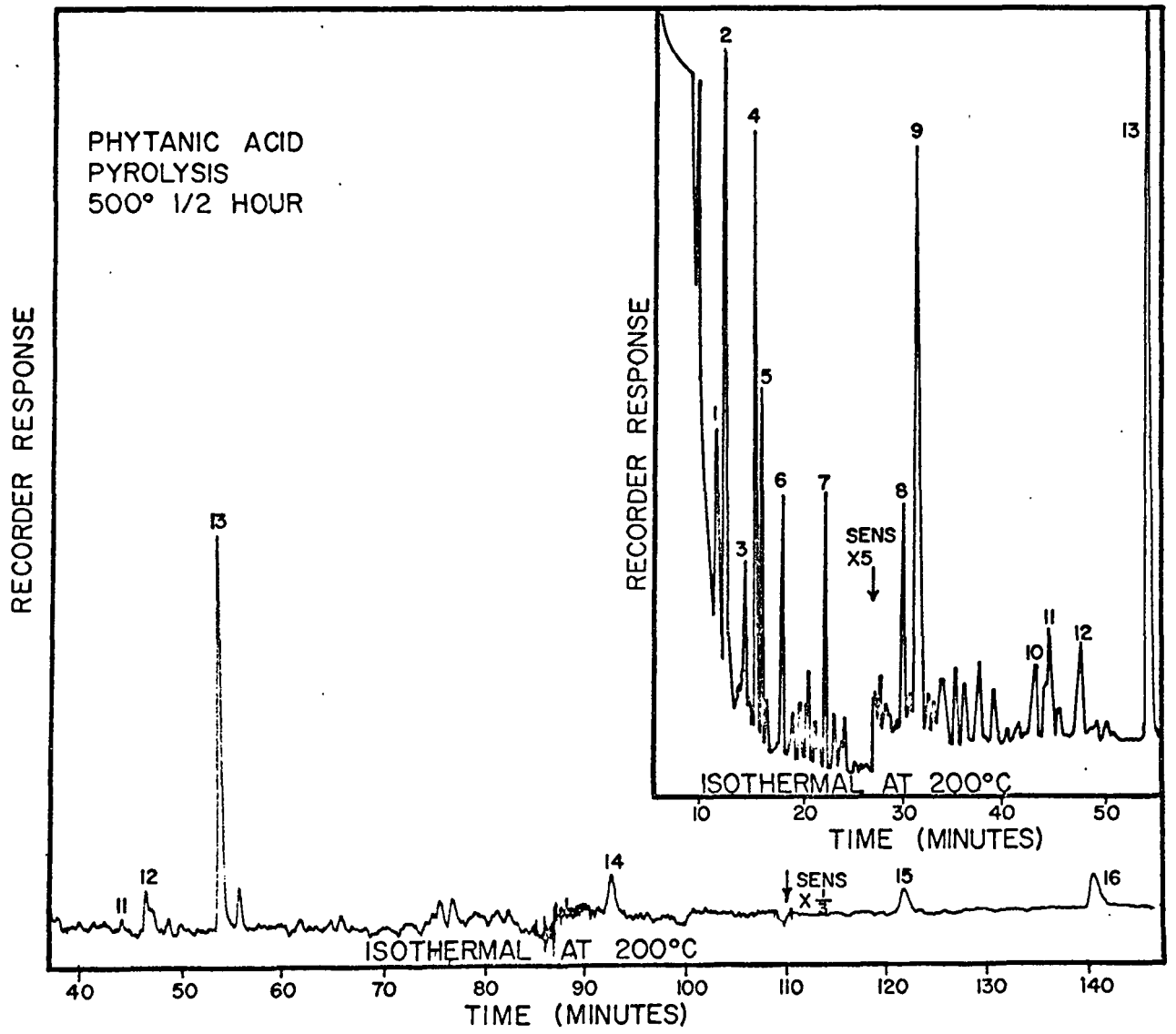
Recorder Span: X 5

Attenuation: 10

Identification:*

1. C_{12}	10. C_{17}
3. C_{13}	11. Pristane
6. C_{14}	14. C_{20}
9. C_{16}	2,4,5,7,8,12,13,15. Unidentified

*tentative


PHYTANIC ACID
PYROLYSIS
500° 1/2 HOUR
RECORDER RESPONSE
RECORDER RESPONSE
SENS X5
SENS X 1/3
ISOTHERMAL AT 200°C
ISOTHERMAL AT 200°C
TIME (MINUTES)
TIME (MINUTES)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
10
20
30
40
50
40
50
60
70
80
90
100
110
120
130
140

Pyrolysis of L-leucine

One µl of a 1% aqueous solution of L-leucine was placed in the liner and heated in an oven at 100°C until the water evaporated. The residue was pyrolyzed at 300°C for 10 minutes in an atmosphere of helium and then the contents of the chamber were swept onto the column by a 10-second flush with carrier gas.

The results are shown in Figure 12. A blank was run under the exact same conditions, and this showed extraneous peaks which are presumably due to the degradation of teflon (Figure 13). Peaks 8, 9, 11, 15 and 16 do not appear on the blank run nor do peaks A, B, and 1-7. Since there is not a smooth distribution between peaks 7 and 8, then the peaks at the upper end of the alanine pyrolysis chromatogram which do not appear on the blank run must have been a result of the teflon reacting with the amino acid at the higher temperatures. Peaks 4-7 could also be a result of the same reaction, but they appear to be homologous and it is felt that each is a true amino acid pyrolysis product. None of the peaks corresponded to any hydrocarbon standards run under the same conditions.

Pyrolysis of L-alanine

One µl of a 1% aqueous solution of L-alanine was treated in the smae manner as L-leucine. The analysis is shown in Figure 14.

FIGURES 12, 13, 14

GAS CHROMATOGRAPHIC ANALYSIS OF

A. THE PYROLYSIS PRODUCTS OF L-LEUCINE

B. AMINO ACID PYROLYSIS BLANK

C. L-ALANINE PYROLYSIS PRODUCTS

Injection: 10-second flush

System: B

Column: SF-96

Temperature: 50°C for 16 minutes, programmed at 3°/minute to 195°C

Recorder Span: X 1

Attenuation: 30

Identification:*

A

A. Amines and Nitriles

B. H_2O

1,3 Unidentified

2. Decarboxylated leucine

4,5,6,7. Homologous series of amines

8-16. Unidentified

10-14. In blank run

B

1. Unidentified (blank)

2. Unidentified (blank)

3. Unidentified (blank)

4. Unidentified (blank)

C

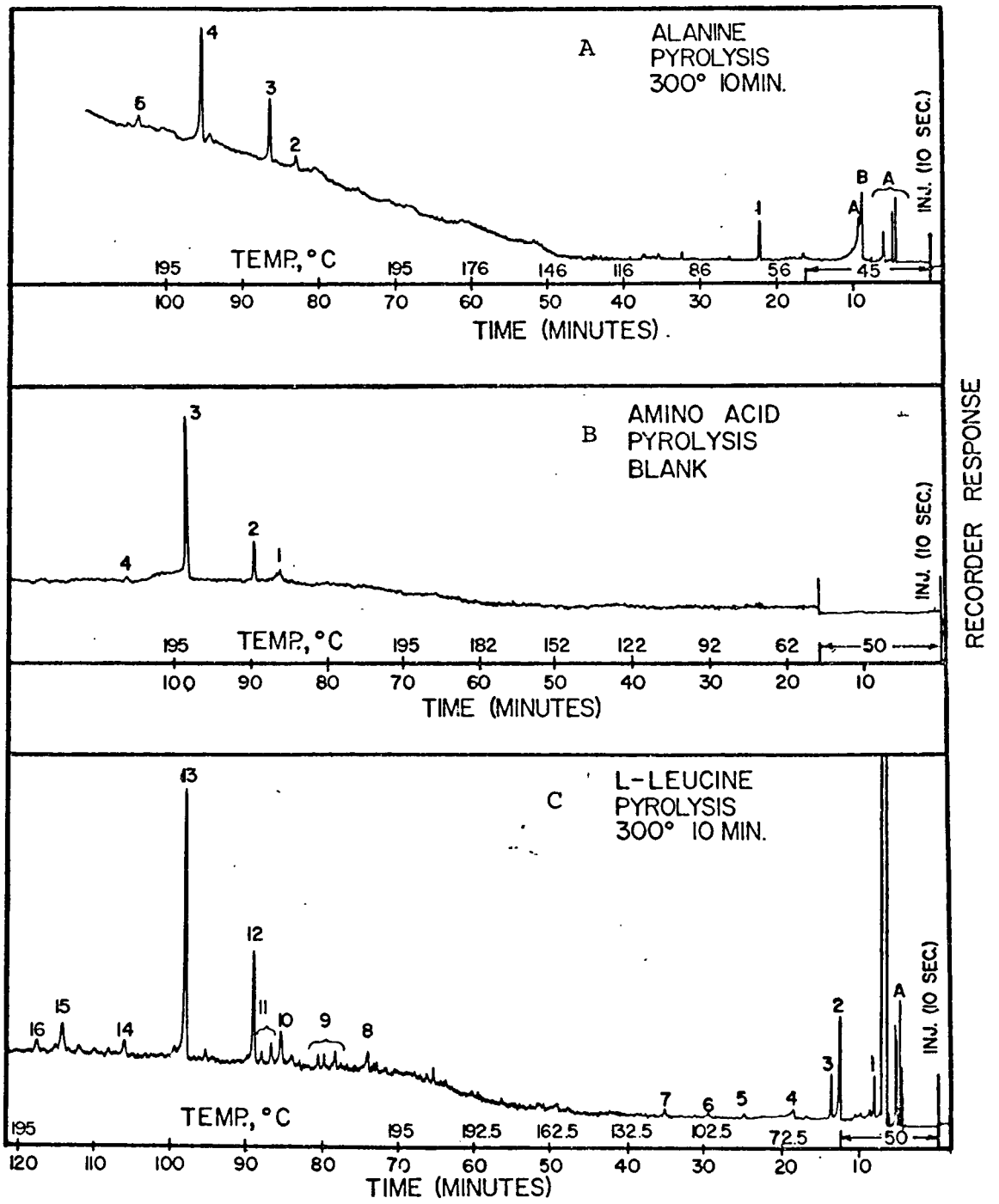
A. Amines and nitriles

B. H_2O

1. Decarboxylated alanine

2,3,4,5. In blank run

*tentative


A
ALANINE
PYROLYSIS
300° 10MIN.
INJ. (10 SEC.)
TEMP., °C
195 100 90 80 70 60 50 40 30 20 10
195 176 146 116 86 56 45
TIME (MINUTES).
B
AMINO ACID
PYROLYSIS
BLANK
INJ. (10 SEC.)
TEMP., °C
195 100 90 80 70 60 50 40 30 20 10
195 182 152 122 92 62 50
TIME (MINUTES)
C
L-LEUCINE
PYROLYSIS
300° 10 MIN.
INJ. (10 SEC.)
TEMP., °C
195 120 110 100 90 80 70 60 50 40 30 20 10
195 192.5 162.5 132.5 102.5 72.5 50
TIME (MINUTES)
RECORDER RESPONSE

IV.

DISCUSSION

DISCUSSION

Pyrolysis of Fatty Acids

The pyrolysis of fatty acids yields a fairly smooth distribution of hydrocarbons with more and less carbon atoms than the original acid. The most abundant paraffin is that represented by n-1 carbon atoms, where n is the number of atoms in the original fatty acid. A plot of relative abundances against carbon number of the paraffin products is shown in Figures 15-18 for the cases of myristic, palmitic and stearic and the mixture of palmitic and stearic.

Jurg and Eisma (13) had also obtained higher molecular weight paraffins in their experiments with behanic acid pyrolysis; the highest abundance of paraffin was that which corresponded to the decarboxylated fatty acid. However, they reported using a sealed system in which some pressure is developed. An examination of Figures 19 and 20, for the sealed system, show high abundances for n-1, although in the case of palmitic, n-2 is preferred. This result is anomalous in comparison with the other pyrolyses; however, all figures show troughs at n, which is in agreement with the rapid initial decarboxylation of the fatty acid.

If the decarboxylation yielded a radical $R\cdot$, and this radical extracted a hydrogen atom from a fatty acid or another intermediate [a mechanism suggested later by Jurg and Eisma (65)], then the most abundant paraffin, at least initially, would contain one less carbon atom than the original acid. Eventually, the system would approach an equilibrium

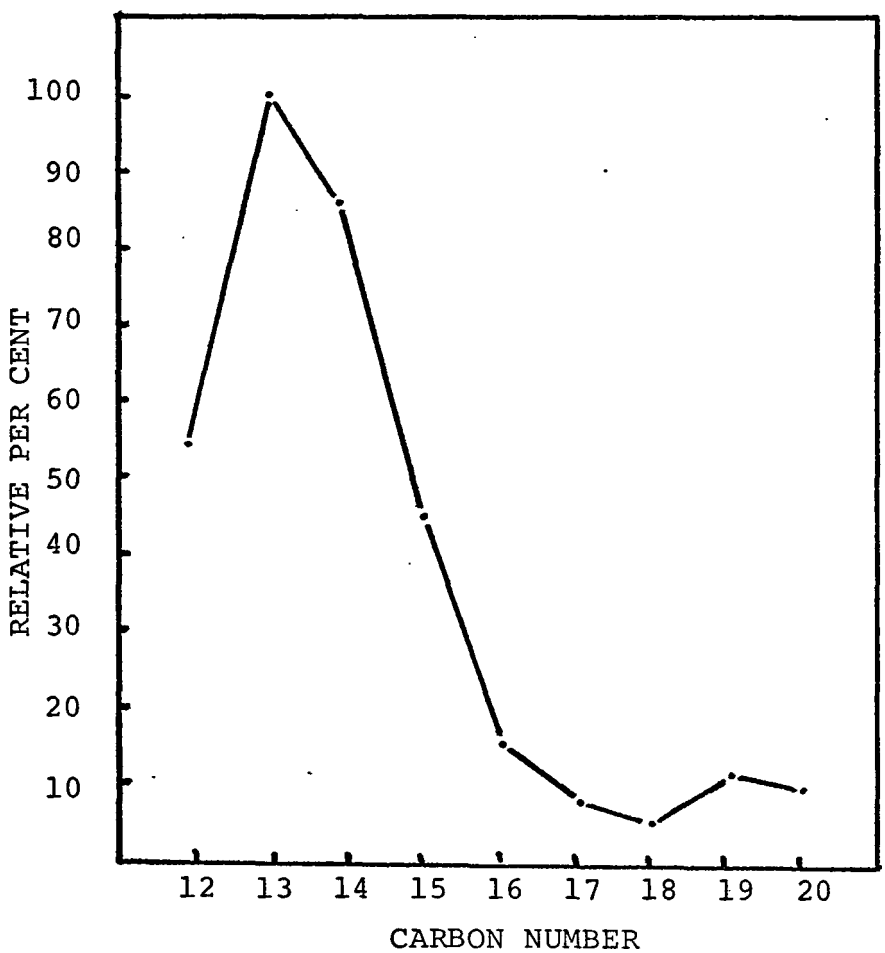


FIGURE 15. THE DISTRIBUTION OF PARAFFINS IN THE PYROLYSIS PRODUCTS OF MYRISTIC ACID

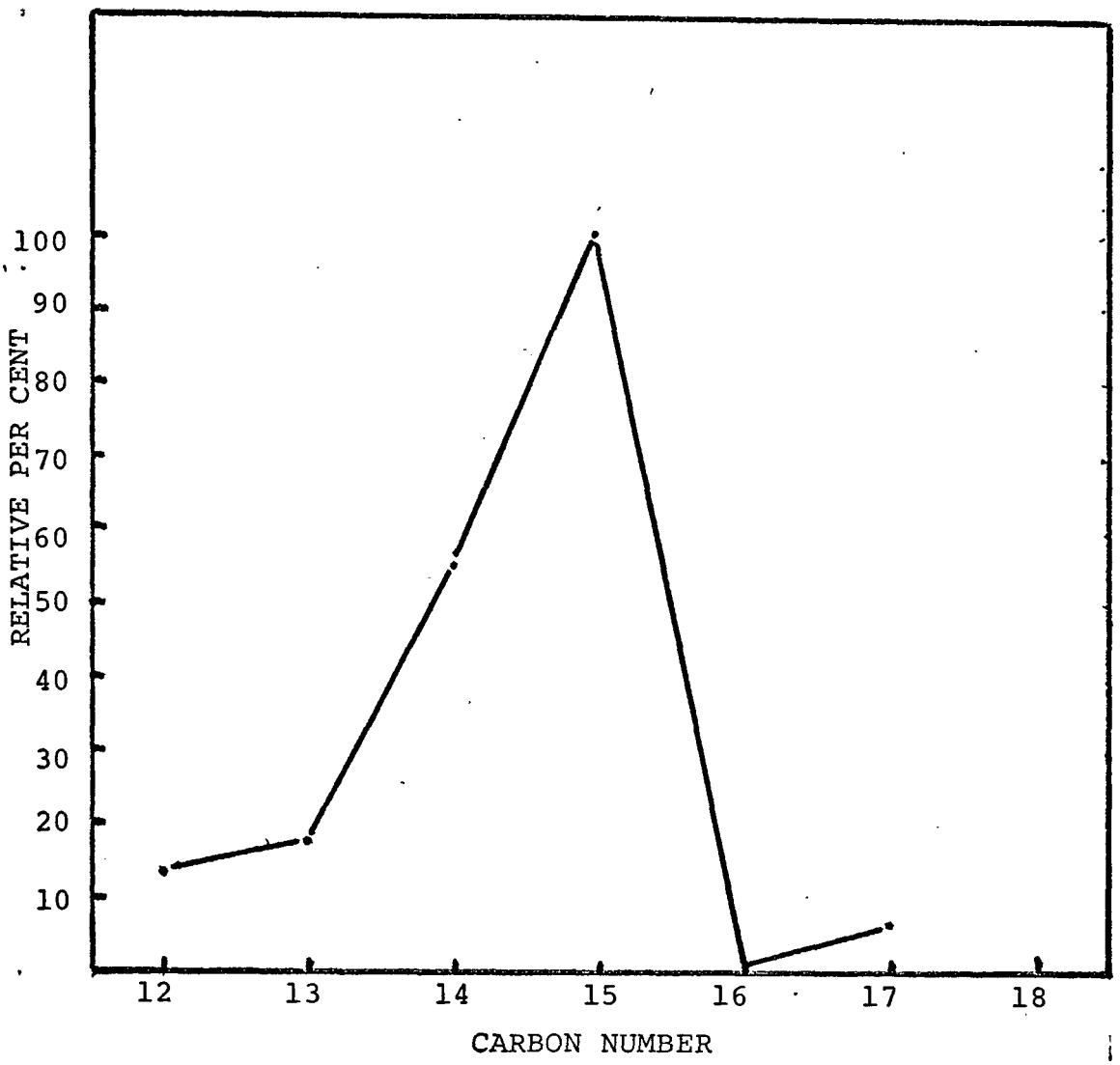


FIGURE 16. THE DISTRIBUTION OF PARAFFINS IN THE PYROLYSIS PRODUCTS OF PALMITIC ACID

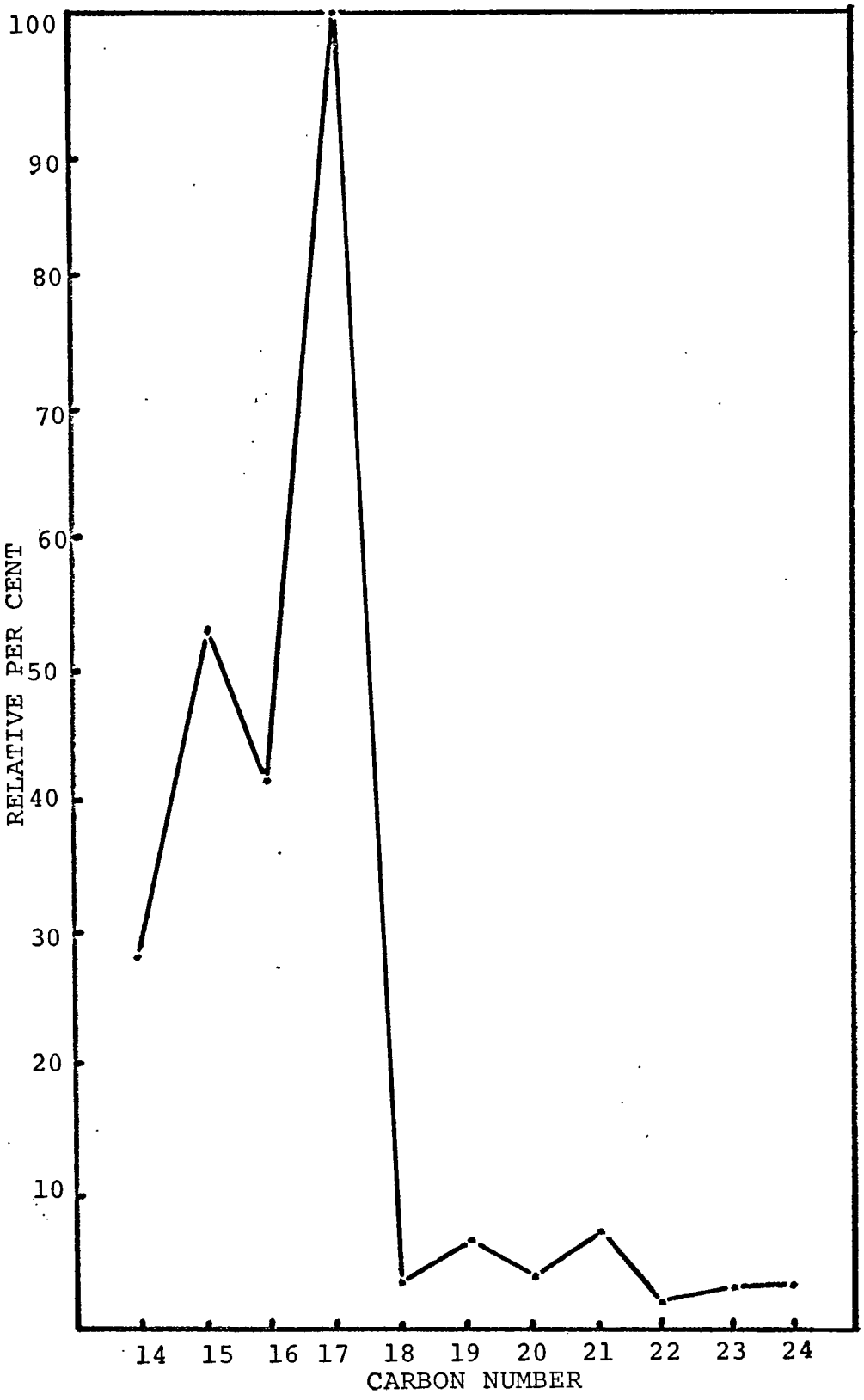


FIGURE 17. THE DISTRIBUTION OF PARAFFINS IN THE PYROLYSIS PRODUCTS OF STEARIC ACID

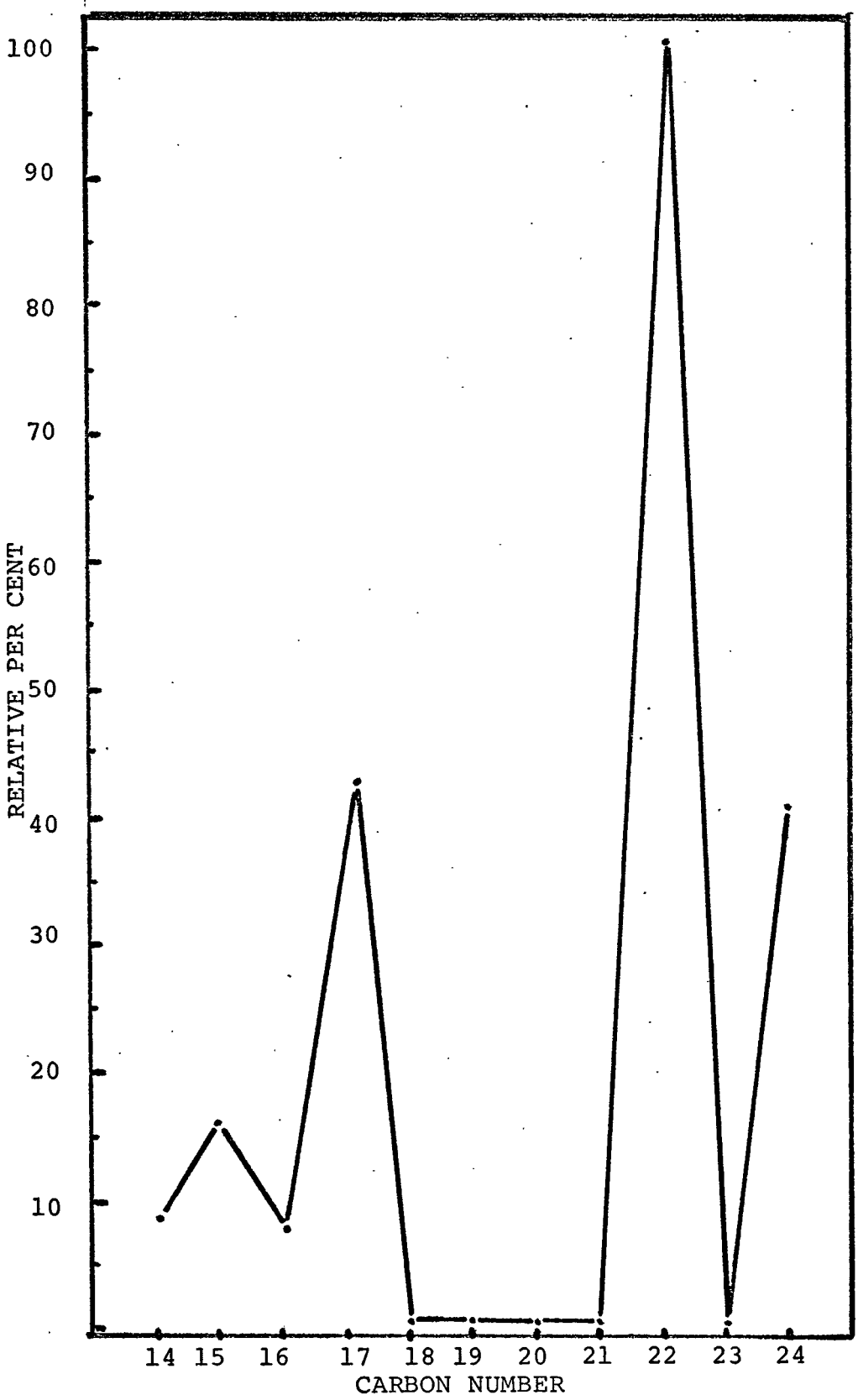


FIGURE 18. THE DISTRIBUTION OF PARAFFINS IN THE PYROLYSIS PRODUCTS OF MIXED FATTY ACIDS

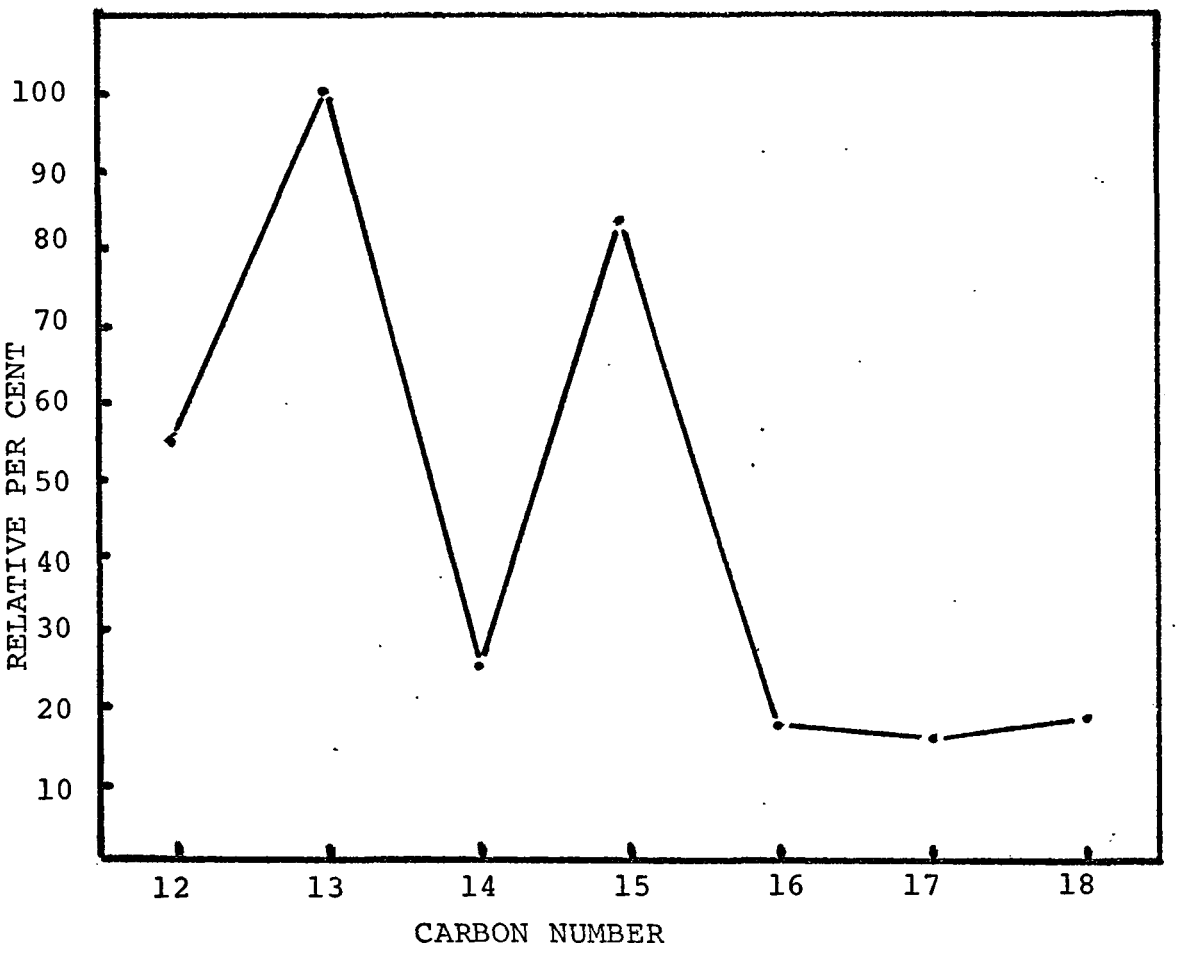


FIGURE 19. THE DISTRIBUTION OF PARAFFINS IN THE PYROLYSIS PRODUCTS OF MYRISTIC ACID (SEALED SYSTEM)

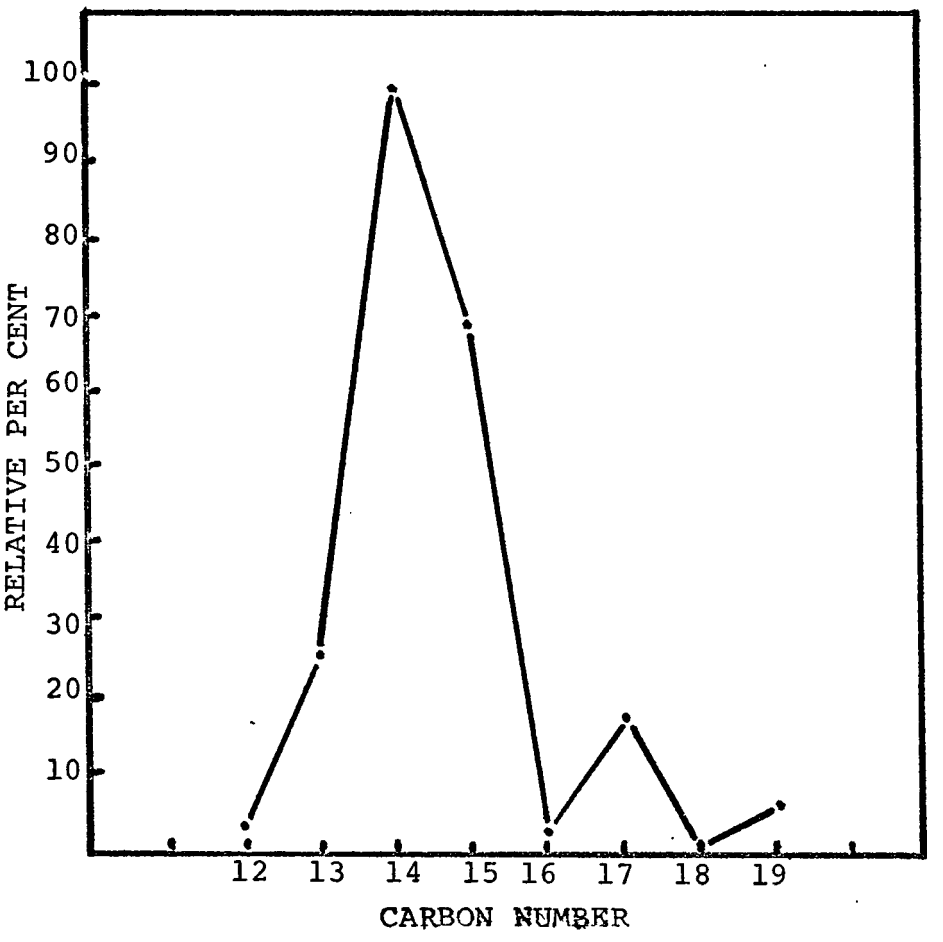


FIGURE 20. THE DISTRIBUTION OF PARAFFINS IN THE PYROLYSIS PRODUCTS OF PALMITIC ACID (SEALED SYSTEM)

in which there is no preference for a particular carbon number.

A smoother distribution as well as some higher molecular weight paraffins might be expected if the duration of the heating time was increased. The pyrolysis of the mixed acids (palmitic and stearic) for two hours at 300° gave the expected predominance of C-15 and C-19 paraffins, as well as an unexpectedly large amount of C-22 and C-24 (Figure 8). These anomalies are probably a result of this particular mixture and a study of similar binary mixtures would have to be undertaken to explain them more fully. Pyrolysis of the mixed acids for four hours at this same temperature gave more of the olefins, but C-22 as well as higher paraffins are not evident. Since olefins are a common product of cracking processes, these conditions are probably too severe for the production of paraffins in major yields.

Repeated experiments with palmitic failed to yield any hydrocarbons above C-19. It is doubtful whether this has anything to do with the intrinsic stability of the acid because the pyrolysis of myristic gave C-20 and the pyrolysis of stearic yielded C-24, six carbon atoms longer than the parent compound. Boldyriv (92) has stated that the previous history of a solid substance and the method by which it is produced affect the rate of its thermal decomposition.

Künkler (70) has stated that the formation of petroleum from degradation of fatty acids proceeds through a ketone intermediate and that pressure hinders rather than promotes this reaction. Connant (72) has said that pressure has no effect on this reaction, while Petrow (12) showed that pressure was necessary in order to produce higher

molecular weight naphthenic components. The pyrolysis of palmitic acid in a sealed system did yield higher molecular weight n-paraffins. However, because of the apparently anomalous behavior of palmitic acid compared with myristic and stearic, the same experiment was repeated with the former. In this case, less higher molecular weight compounds were observed with the sealed system. What is more important is that in the case of both acids, much larger abundances of n-1 carbon numbered paraffins were seen compared with others. This plus the fact that large amounts of acid were left unreacted at the end of the two hour heating time indicates that pressure hinders the reaction.

Pyrolysis of the Methanol Fraction of Bacillus cereus

The analysis of this pyrolysis shows large amounts of olefins in comparison to the saturated hydrocarbons. This is in contrast to the experiments with the individual acids but is not surprising in view of the fact that this organism contains substantial amounts of unsaturated fatty acids. Although Kates (93) in discussing the fatty acid components of B. cereus, gives evidence for a large amount of saturated C-17, the decarboxylation product, the C-16 alkane does not seem to be commensurately high. Abelson (67), in examining the thermal stability of the fatty acid components of algae states that while the saturated and the mono-unsaturated acids are about equally resistant, the saturates are apparently more enduring. This and the fact that there are other unsaturated components, notably C-16, would account for this phenomenon,

especially the unsaturated C-16 hydrocarbons. It is interesting to note that some pristane, the decarboxylation product of phytanic acid, was obtained.

Pyrolysis of Phytanic Acid

Although some pristane was produced in this pyrolysis, it was by no means the major product. The difficulties in obtaining suitable conditions have already been mentioned. While lower pyrolysis temperatures would probably be less severe, a much longer heating time would be required. Certain paraffin hydrocarbons were obtained, largely C-12, C-14, C-16 (the unbranched, reduced skeleton of phytanic acid), in addition to C-17 and C-20. Although no positive identification of the major components was accomplished, it would not be unreasonable to assume that they were branched chain hydrocarbons. In fact, Curphey (58) actually postulated a mechanism utilizing thermal cracking to produce a host of intermediates suitable for geosynthesis of branched-chain alkanes found in petroleum, such as the 2-methyl alkanes. Mass spectrometric identification of the pyrolysis products of phytanic acid would be a rapid means of determining these intermediates.

In the Introduction, two proposed mechanisms were given for the degradation of phytol into petroleum precursors, one via the decarboxylation of the phytanic acid intermediate, the other by way of hydrogenation and dehydration of phytol. This second reaction was inadvertantly performed in the laboratory in trying to synthesize phytanic acid (see

Experimental). Large amounts of phytane were produced, in higher yield even than phytanic acid (Fig. 21). However, in addition, some pristane was produced as well as oxygen containing compounds (presumably ketones or aldehydes). The amount of pristane was increased during methylation and during a preliminary pyrolysis of the impure phytanic acid at 300°C. This is, in effect, hydrogenolysis followed by thermal degradation of the intermediate compounds. It would lead to the conclusion that petroleum precursors are available directly from phytol, in addition to the geosynthetic pathways proposed by Curphey and Bendoraites et al. (58,76).

Pyrolysis of Amino Acids

In the two cases attempted, no hydrocarbons were found, and certainly none of higher molecular weight. The highly volatile components would undoubtedly be amines, corresponding to the amines produced in other experiments (87,88). The difficulty is not with the decarboxylation, but rather with reductive deamination. While Erdman (50) has stated that such a step must take place because of the presence of ammonia in petroleum source rocks where amines were not detected, he gives no evidence of such a reaction having been performed. In addition, the susceptibility of amino acids to bacterial destructions renders them improbably precursors of hydrocarbons through geochemical transformation. Cases where amino acids were thought to have survived this destruction have later proved them to be adventitious (94).

FIGURE 21

GAS CHROMATOGRAPHIC ANALYSIS OF METHYL PHYTANATE REACTION MIXTURE

Injection: 1 µl

System: C

Column: Polysev

Temperature: 140°C for 10 minutes, programmed at 2°/minute to 180°C

Range: 100

Attenuation: 1

Identification:

1. Pristane
2. Phytane
3. Unidentified
4. Unidentified
5. Methyl Phytanate

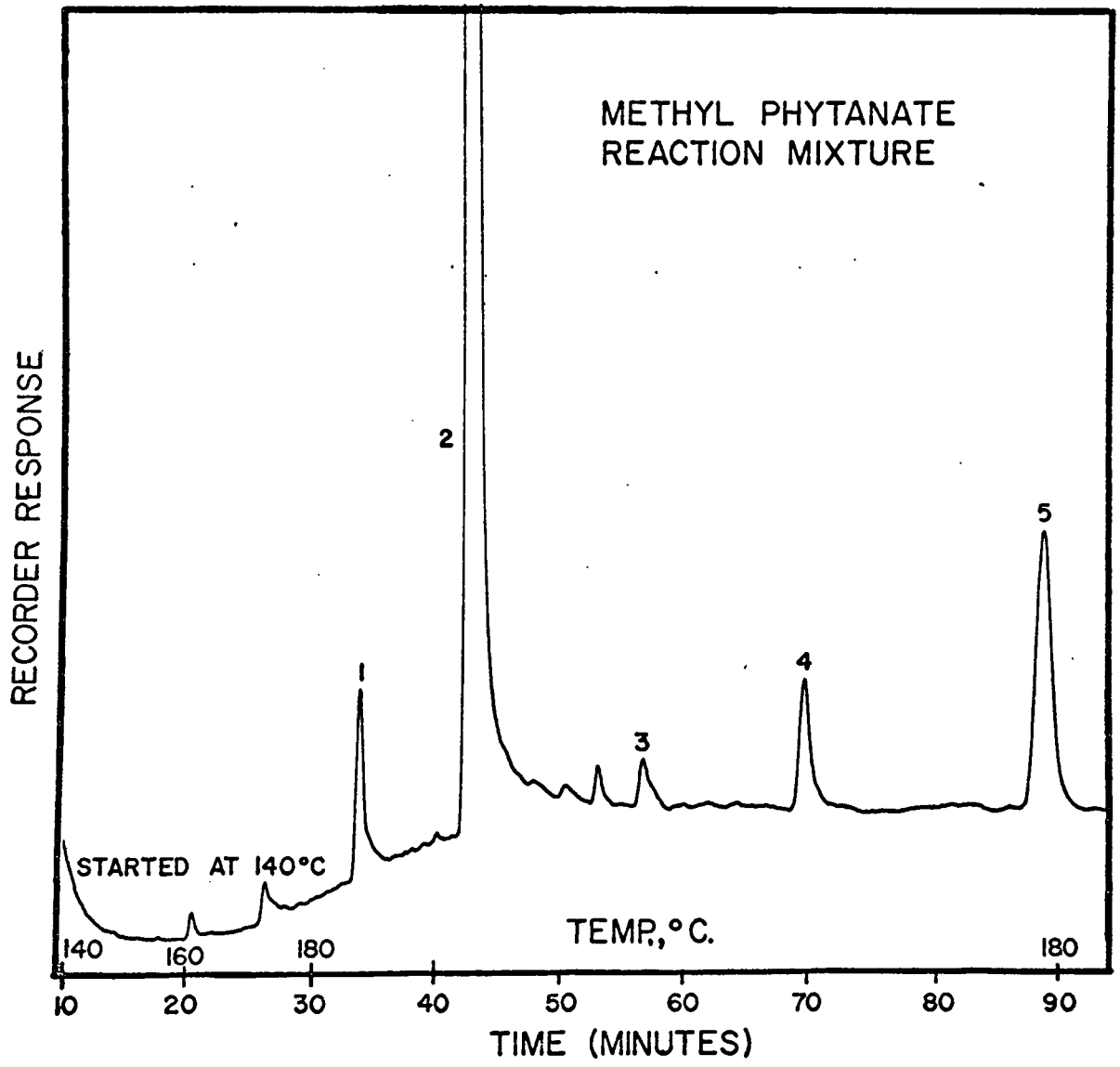


FIGURE 21. GAS CHROMATOGRAPHIC ANALYSIS OF METHYL PHYTANATE REACTION MIXTURE

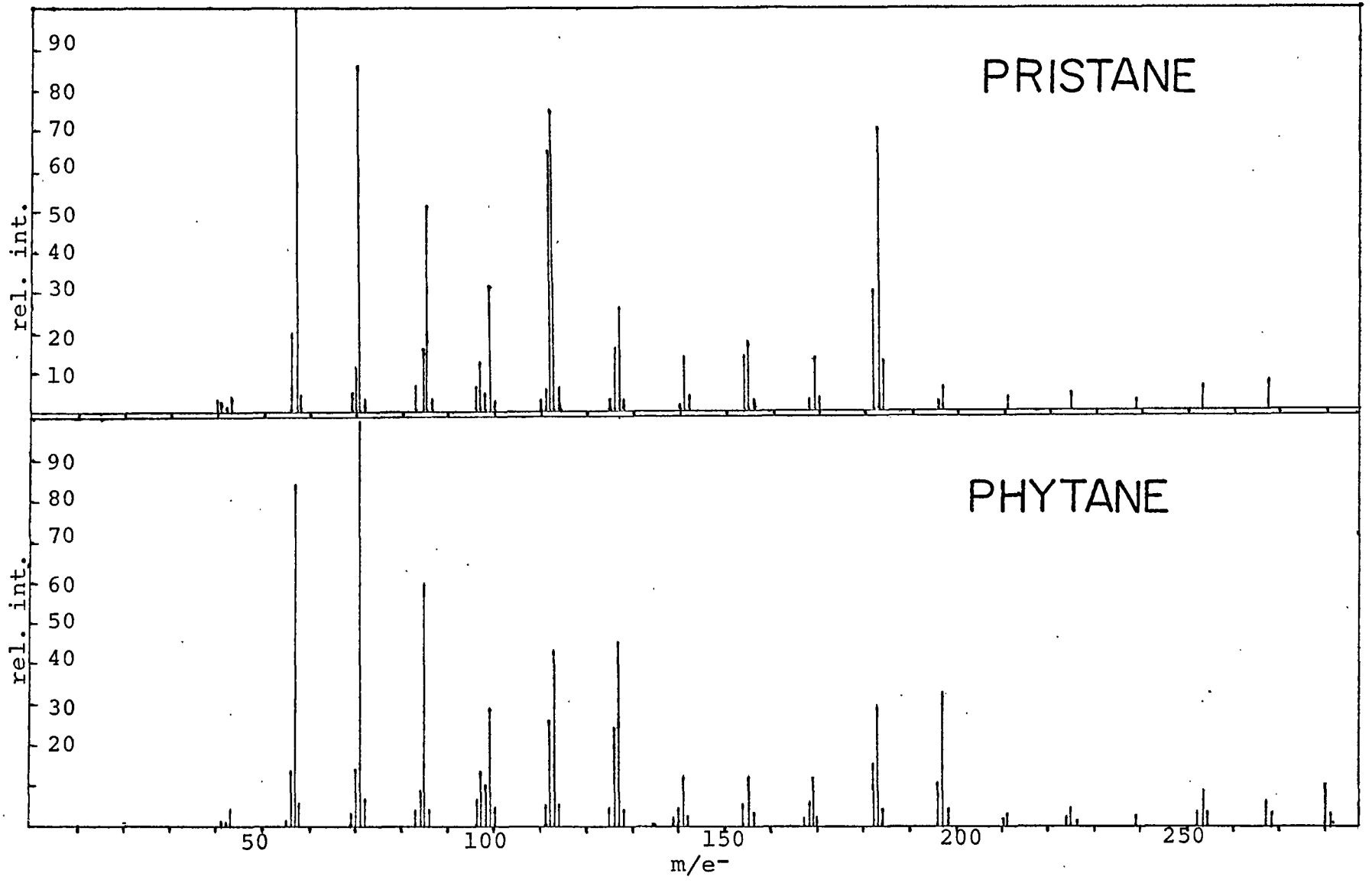


FIGURE 22. MASS SPECTROMETRIC IDENTIFICATION OF PRISTANE AND PHYTANE

V.

CONCLUSIONS

CONCLUSIONS

Investigations of sediments, crude oils and petroleum reservoirs have established a correlation between hydrocarbons found therein and naturally occurring fatty acids. These investigations, and the comparison of predominances and distribution of these two substances, have led to the conclusion that petroleum hydrocarbons arise, at least in part, from fatty acids and that decarboxylation of the latter could account for the unique distribution of the former.

The data obtained from the accelerated decarboxylation of fatty acids supports this conclusion in the following ways:

1. The major product obtained from most pyrolysis was the decarboxylated hydrocarbon. Even the olefins that were identified showed a preference for carbon number corresponding to one less than the pyrolyzed fatty acids. The distribution of fatty acids is predominantly even in nature with an apex at C-16 and C-18. Decarboxylation of these acids would give a similar distribution of hydrocarbons, an almost universal observation in recent sediments.
2. Carbon numbers of hydrocarbons produced by these pyrolyses exceeded the carbon number of the original fatty acid, in some cases as much as six, and were found in major yields.

The differences between odd and even carbon number were not dramatic for these higher molecular weight compounds, a situation which compares favorably with the investigations of ancient crudes.

Moreover, since similar results were obtained with mixed fatty acids and the methanol fraction of a bacterium, it is quite likely that fatty acids, which would give rise to a substantial amount of petroleum hydrocarbons, could have been deposited by living organisms. On the other hand, the amino acids are probably not an important source. Since pristane and phytane, as well as a host of other branched alkanes, have been isolated from petroleum, it is probable that phytol and/or phytanic acid could have supplied these. Pyrolysis of phytanic acid gave pristane and some n-alkanes. The hydrogenolysis of phytol yielded pristane and phytane.

Pyrolysis under pressure gave results which indicated that the reaction has not proceeded as far as it would have had the pressure been reduced. In air, however, the reactants charred, although a distribution comparable to the other pyrolyses was obtained.

Suggestions for Future Work

1. Cooper (52) and Jurg and Eisma (65) have proposed mechanisms for the geosynthetic transformations of fatty acids into petroleum hydrocarbons in which fatty acids of longer and shorter chains than the pyrolyzed acid are intermediates. These could be detected by the methods used here. The methanol extract of the pyrolysis residue is methylated by a procedure suitable for small quantities (95) and examined by gas chromatography.

2. An increase in heating time was undertaken with the mixed acids, primarily to determine a convenient reaction time. Some higher

molecular weight hydrocarbons were obtained but the distribution was different than the pyrolysis for two hours. Therefore, it is suggested that a detailed study of the effects of both time and temperature be made with respect to pyrolytic transformations of fatty acids. It has already been recognized that a change in reaction temperature of a few hundred degrees could effect transformations in a few hours that geochemically might have taken billions of years.

3. The pyrolysis products of phytanic acid and phytol should be analyzed by combined gas chromatographic-mass spectrometric techniques. The results should be compared with what is already known about the distribution of branched alkanes in petroleum hydrocarbons, especially the 2-methyl alkanes and the isoprenoid isoalkanes (96,97).

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