A study of the hydrocarbons of the cuticular wax of Anabrus simplex Hald
by Roger John Leibrand

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Chemistry at Montana State College
Montana State University

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The hydrocarbons in the lipid solvent extractable material from the cuticle of Anabrus simplex Hald. have been examined in an effort to elucidate their type and relative amounts. Alumina column chromatography was used to separate the hydrocarbons from the other wax components. Gas chromatography was used for further separation and identification. Molecular sieve adsorption was used to differentiate between normal and branched hydrocarbons. Identification of certain hydrocarbons collected with the gas chromatograph utilized infrared spectroscopy, nuclear magnetic resonance spectrometry, and mass spectrometry. A series of normal hydrocarbons from C10 through C35 (except C35), a probable series of iso hydrocarbons from Cll through C27, and two anteiso hydrocarbons, C28 and C30, were found. Other branched hydrocarbons of higher molecular weight were found but not definitely identified.

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March, 1962

ACKNOWLEDGEMENTS

I wish to thank Dr. Graeme L. Baker for his guidance and encouragement while I have been doing this research.

My thanks also go to Mr. Ellsworth Hastings and his associates for their part in the collection of the crickets and the preparation of the cuticles.

I wish to thank Mr. Roy Johnson of Varian Associates who ran the N.M.R. spectra of some hydrocarbons and Mr. Seymour Meyerson and Mr. E. B. Tucker of the American Oil Company who obtained the mass spectral analyses of these compounds.

Mr. Joseph Dixon of the American Petroleum Institute also receives my thanks for furnishing some pure hydrocarbon samples.

My special thanks go to the National Science Foundation for a fellowship that helped immeasurably.

I also wish to thank my wife Kay for her encouragement that was especially needed these past two years.

And this thesis is gratefully dedicated to my parents.

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ABSTRACT

The hydrocarbons in the lipid solvent extractable material from the cuticle of Anabrus simplex Hald. have been examined in an effort to elucidate their type and relative amounts. Alumina column chromatography was used to separate the hydrocarbons from the other wax components. Gas chromatography was used for further separation and identification. Molecular sieve adsorption was used to differentiate between normal and branched hydrocarbons. Identification of certain hydrocarbons collected with the gas chromatograph utilized infrared spectroscopy, nuclear magnetic resonance spectrometry, and mass spectrometry. A series of normal hydrocarbons from C_{10} through C_{36} (except C_{35}), a probable series of iso hydrocarbons from C11 through C_{27}, and two anteiso hydrocarbons, C_{28} and C_{30}, were found. Other branched hydrocarbons of higher molecular weight were found but not definitely identified.

INTRODUCTION

The moisture balance of any living organism is of crucial importance. This is especially true for those animals populating the arid climates. The Mormon Cricket, living in Montana, is one of these animals. The lipids in an insect's exoskeleton have been shown to possess the factors controlling the water balance between the insect and its environment[1]. This waxy layer also is the barrier through which contact poisons must pass in order to affect the insect.

An overall study of the cuticular wax of Anabrus simplex Hald. was done by Baker, Pepper, Johnson, and Hastings[2]. The original selection of this insect was based on economic importance, availability, large size, and the presence of a good waxy protective layer. Their work showed this wax to contain hydrocarbons, free fatty acids, esters, acidic resins, and possibly cholesterol. Specifically they found hydrocarbons made up 48% to 58% of the cuticular wax. The individual components were C_{12} to C_{26} inclusive, C_{27} and/or C_{28}, C_{30} and/or C_{31}, and C_{32}. Relative amounts were also given.

From this general study it was apparent that more work could certainly be done in identifying these hydrocarbons. With modern analytical instrumentation a clear and logical development of the study of the hydrocarbons of the Mormon Cricket is possible. Since hydrocarbons have been found by other workers in various insect waxes[3, 4, 5, 6, 7], it is likely that this study would shed some light on the composition of insect cuticular waxes in general.

PROCEEDURE

Collection of Wax and Initial Separations

The crickets were collected in the summers of 1959 and 1960 from various bands in Montana. These insects were brought in live from the field in cages and were immediately killed by placing them in a closed container filled with chloroform vapor. Until the cuticles were excised, the crickets were kept frozen. The cuticles were removed, cleaned of foreign matter by careful brushing under water, and kept frozen until extraction. No selection was made as to sex and only adults past the fifth molt were used.

The cuticular wax was extracted from the excised abdominal sclerites with a Soxhlet apparatus. Fifty four and nine tenths grams of cuticles were divided into three parts. Each portion was extracted with 2.5 liters of chloroform. The total yield from the three extractions was 1.4822 grams of a wax designated as Wax K. Figure 1 shows the extraction scheme and Figure 3 is an infrared spectrogram of Wax K.

Following the chloroform extraction, Wax K was further extracted with one liter of n-hexane. The insolubles, Wax K_{CR}, were removed by filtration through a one inch filter stick with fibreglass pads. The residue was taken up in chloroform and similarly filtered. The residue here, "Wax K_R", was a solid inorganic material and weighed 0.0193 grams. Being insoluble it was classed as nonlipid. The infrared spectrum of "Wax K_R" indicated it

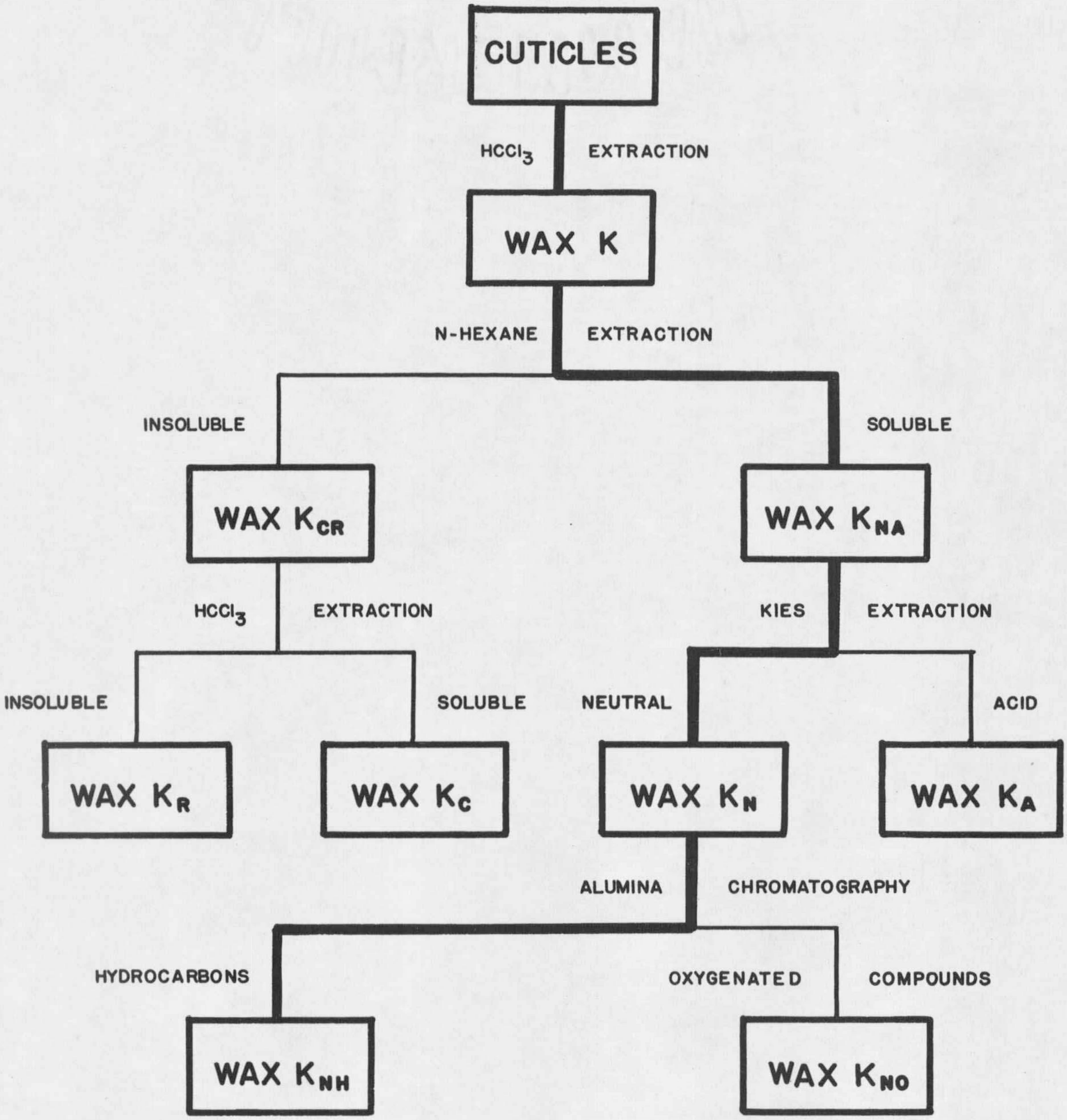


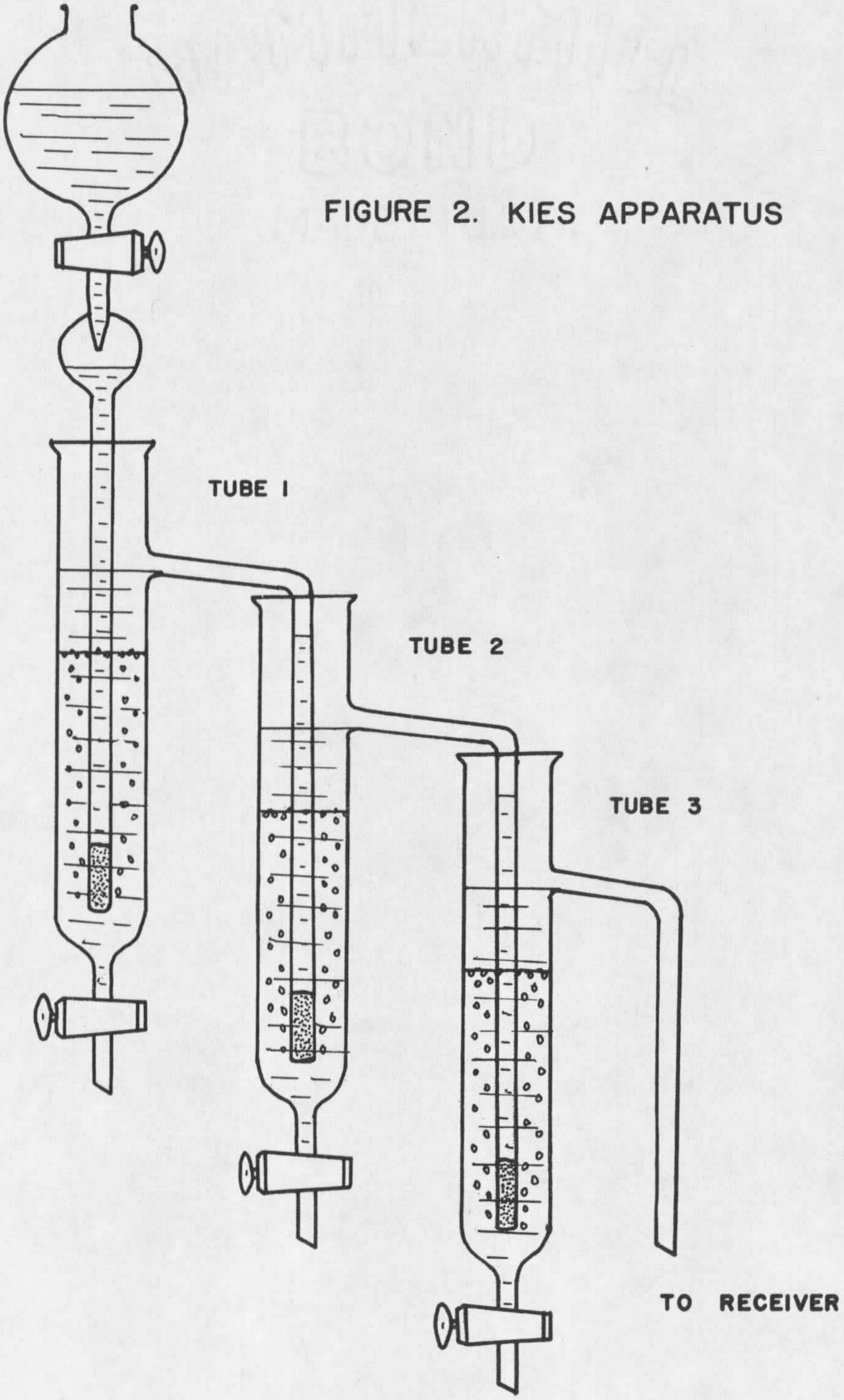
FIGURE I. EXTRACTION SCHEME

was probably a silicate[8]. The solvent was removed from the chloroform soluble Wax K_C with a Rinco rotary evaporator and an infrared spectrogram taken. Wax K_C had resinous characteristics and the spectrogram indicated a complex hydroxy-ester composition[8]. These n-hexane insolubles were set aside for future study by other workers.

The infrared spectrogram of Wax K_{NA}, the n-hexane solubles shows this wax to probably contain hydrocarbons, esters, and acids[8]. The neutrals were separated from the acids by a Kies countercurrent extraction assembly[9]. See Figure 2. Wax K_{NA} in one liter of n-hexane was dripped slowly through the apparatus. Tube 1 contained a mixture of 30 ml. of 95% ethanol, 70 ml. of water, and 10 ml. of 0.1 N KOH. Tube 2 contained a mixture of 30 ml. of 95% ethanol, 70 ml. of water, and 5 ml of 0.1 N KOH. Tube 3 contained a mixture of 30 ml. of 95% ethanol and 70 ml. of water. This particular arrangement was used in order to insure complete removal of the acids as their potassium salts. The aqueous fractions, which contained these salts, were combined, acidified with H_2SO_4, and extracted four times with diethyl ether in 500 ml., 250 ml., 100 ml., and 100 ml. portions. The combined ether extract was then scrubbed twice with 500 ml. and 300 ml. portions of water. This ether extract was then dried over Na_2SO_4 prior to solvent removal on a Rinco evaporator. An infrared spectrogram of Wax K_A was taken and it was indicative of long chain carboxylic acids[8].

The n-hexane soluble fraction that had passed through the Kies apparatus was placed over Na_2SO_4. This solution was then filtered to remove any grains of the drying agent and then the solvent was removed with a

FIGURE 2. KIES APPARATUS



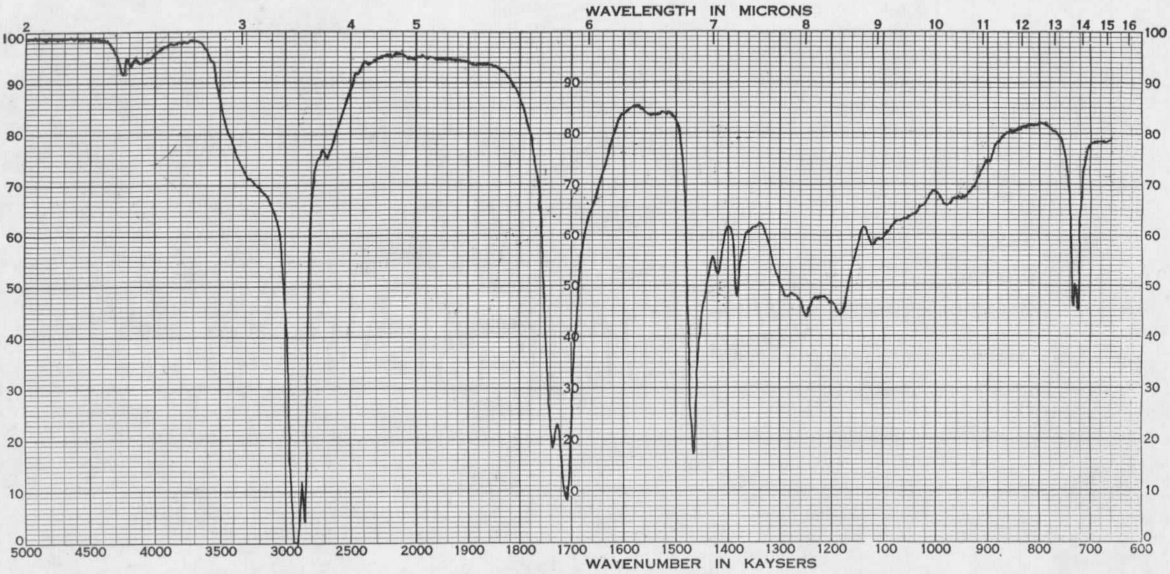


FIGURE 3. INFRARED SPECTROGRAM OF WAX K

FIGURE 4. INFRARED SPECTROGRAM OF WAX K_N

Rinco evaporator. A total of 0.9985 grams of Wax K_N was obtained. The infrared spectrogram of Wax K_N, Figure 4, shows that this fraction may contain hydrocarbons, esters, and hydroxy compounds[8].

Column Chromatography

In order to separate the hydrocarbons from the oxygenated compounds alumina column chromatography was investigated. Drawing from the work of Weidenhof[10], White et al[11], Baker[12], and Houle[13], a method was developed to separate the hydrocarbons from the esters and hydroxy compounds. Thirteen cm. of a 30 cm., 16 mm. diameter pyrex column, fitted with a Teflon stopcock, was filled with Woelm Activity Grade I neutral alumina. The wax was introduced onto the column with a minimum amount of petroleum ether. Five ml. fractions were collected every ten minutes on an automatic fraction cutter. The wax was batch eluted by collecting 25 tubes eluted with 100% petroleum ether, 20 tubes eluted with 4% benzene in petroleum ether, 20 tubes eluted with 4% diethyl ether in petroleum ether, and 25 tubes eluted with 4% acetic acid in petroleum ether. Figure 5 is the elution diagram of this separation. The solvent was removed under reduced pressure from the fraction tubes with a heated orbital shaker. The wax components eluted with the acetic acid solution (tubes 70 through 95), which had a combined weight of 0.4394 grams, were labeled Wax K_{NO} and set aside for other workers. Infrared spectrograms were taken of the several major peaks (tubes 3, 5, and 49) and only compounds of hydrocarbon character

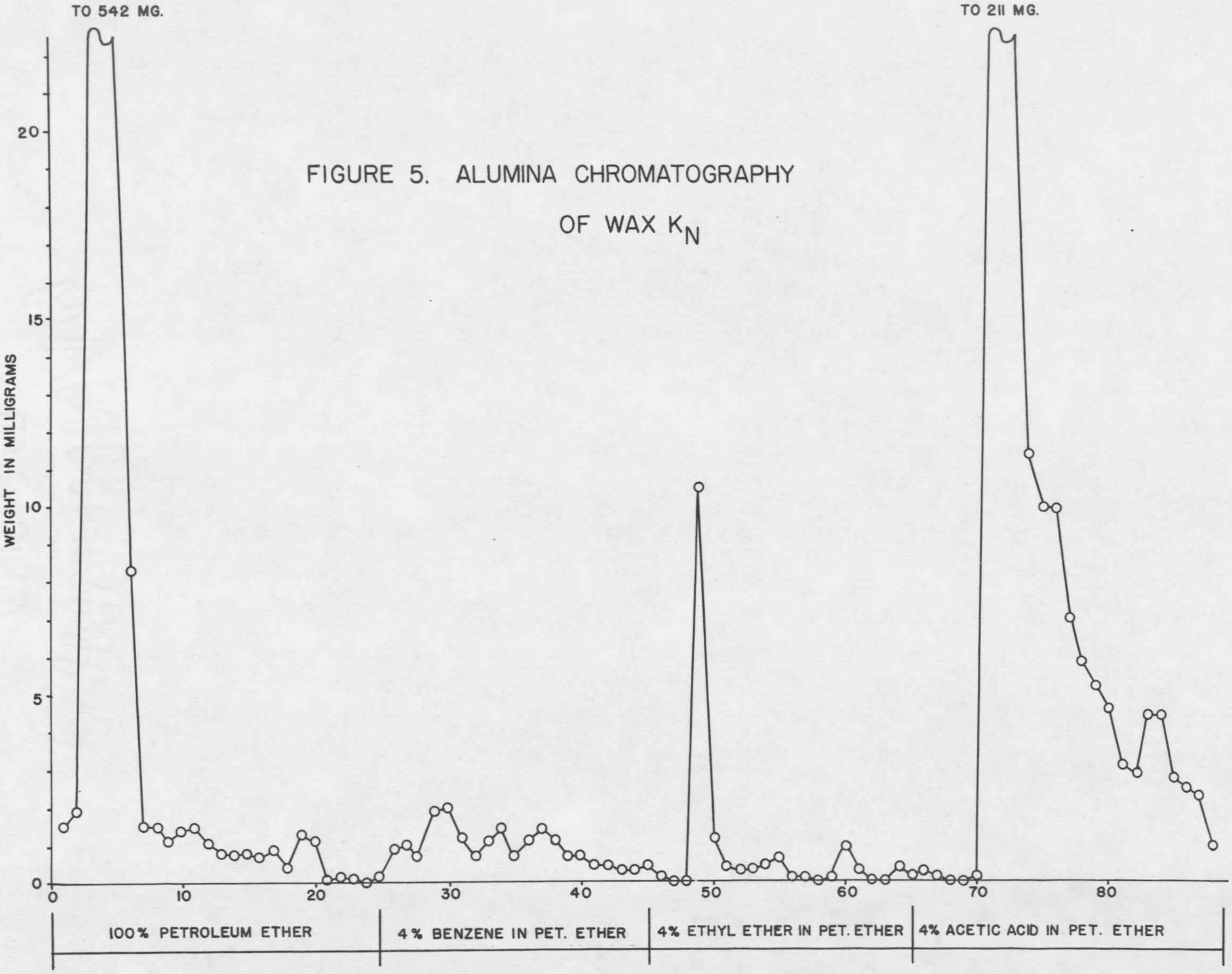


FIGURE 5. ALUMINA CHROMATOGRAPHY OF WAX K_N

were indicated[8]. The remaining 0.5591 grams of wax was designated Wax K_{NH} and consisted of tubes 2 through 69. These hydrocarbons constituted 39% of the cuticular wax of the Mormon Cricket.

Gas Chromatography

All of the gas chromatography was done on a Wilkens A-90C Aerograph. This instrument utilizes a hot wire thermal conductivity detector in a bridge arrangement. The carrier gas was helium and a 1 mv. Brown recorder was used. The gas chromatograph was used for compound identification and collection of pure samples. Many different column packings, column lengths, and temperatures were tried. The conditions for optimum separation depended upon the compounds studied. Two hundred and forty degrees Centigrade was good for hydrocarbons up to C_{25} and 265° C. was good up to C_{40}. Any one temperature setting was good only for a certain range of compounds. The peaks of the lighter ones were run together and the peaks of the heavier ones were too spread out. Most work was done at 265°C., 60 ml./min. of helium flow (calculated from height equivalent to a theoretical plate values[14]), with an 11 foot, 1/4 inch inside diameter copper column filled with 15% silicone rubber on 30/60 mesh Johns-Manville Chromosorb.

Molecular Sieves

Much work was performed using Linde MS-5A (5 Å opening) molecular sieves. Adsorption of normal hydrocarbons from liquid solutions was attempted with no great success after the method of O'Connor and Norris[15]. The gas chromatographic method of Whitham[16] was then tried. It consisted of fitting a regular gas chromatography column with a pre-column of molecular sieves. An 11 foot, 1/4 inch inside diameter column was packed with 10 feet of 15% silicone rubber on 30/60 mesh Chromosorb and one foot of Wilkens ball-form molecular sieve in the fore-part of the column. This method proved very effective for the removal of the normals from hydrocarbon mixtures. It should be noted that the molecular sieves must be heated to about 600°C. with a small current of dry air blown over them for at least 4 hours to remove any water present.

Infrared Spectroscopy

All of the infrared spectrograms were obtained with a Beckman IR-4 with NaCℓ optics. The spectra were taken on pure materials (capillary films and melts) and liquid solutions ($CC\ell_4$ solvent in a 0.1 mm. cell).

Nuclear Magnetic Resonance Spectrometry

Several of the major components of Wax K_{NH} were separated and collected by gas chromatography. The exit port of the gas chromatograph was fitted with a U-tube collector packed with washed cotton and wetted with carbon tetrachloride. After collection the hydrocarbons were washed from the cotton and found to contain silicone rubber and/or silicone rubber decomposition products[8]. These were removed by eluting the mixture through a 3/8 inch by 10 inch Woelm Activity Grade I neutral alumina column. The solvent was then partially removed and the hydrocarbons put in sealed containers and sent to Varian Associates for N.M.R. analysis. The purpose of this analysis was a proton count to determine the ratio of methyl hydrogens to methylene hydrogens.

Mass Spectrometry

With the number of branches on the hydrocarbon molecule determined from N.M.R. data it was now necessary to determine the size and location of these branches. Mass spectrometry is ideally suited to the solving of this problem. Chromatographically pure samples (the same ones used for the N.M.R. analysis) were then prepared for mass spectral analysis. The hydrocarbons in benzene solution were sent in sealed containers to the American Oil Company for analysis on their high mass instrument.

Chemical Tests

Tests were made on 0.05 gram samples of Wax K_{NH} to determine if there was any unsaturation present. Conditions were used that favored the addition of bromine rather than substitution. Gas chromatograms were taken of the wax before and after the test. In no case was any peak observed to be diminished, removed, or altered in retention time. This same test was also performed using the molecular sieve pre-column and no different results were noted. Known unsaturates were also brominated to check this method. No unsaturated hydrocarbons were found in Wax K_{NH} so further work along this line was unnecessary.

ANALYSIS OF DATA

Initial examination of Wax K and its infrared spectrogram would not lead one to the conclusion that hydrocarbons are present. Neither would examination of Wax K_{NA} nor Wax K_N. However, since it was known that hydrocarbons were present in these waxes, alumina chromatography was undertaken. The hydrocarbons were separated from the neutral oxygenated compounds by this proceedure. Figure 5 is the elution diagram of this proceedure. The 100% petroleum ether eluted the majority of the hydrocarbons. Figure 6 is an infrared spectrogram of the contents of tube 5. It indicates that the sample is completely hydrocarbon in character as expected. With an eluting agent of slightly more polarity, 4% benzene in petroleum ether, more hydrocarbons were eluted. Their infrared spectrogram shows no discernable difference from that of tube 5. Likewise, the more polar 4% diethyl ether in petroleum ether eluted more hydrocarbons whose infrared spectrogram again shows no difference from that of tube 5. On this basis tubes 2 through 67 were combined and labeled Wax K_{NH}.

A portion of Wax K_{NH} was tested for unsaturation. The result was negative. This proceedure is outlined on page 17. It was now known that Wax K_{NH} contained only saturated hydrocarbons.

Examination of the infrared spectrum of Wax K_{NH} was some help at this point in further determination of the character of the hydrocarbons present. The prominent peak at 720 cm^{-1} is indicative of long chain hydrocarbons and the breadth of the 1380 cm^{-1} and 1470 cm^{-1} peaks indicates the possibility of branching[8].

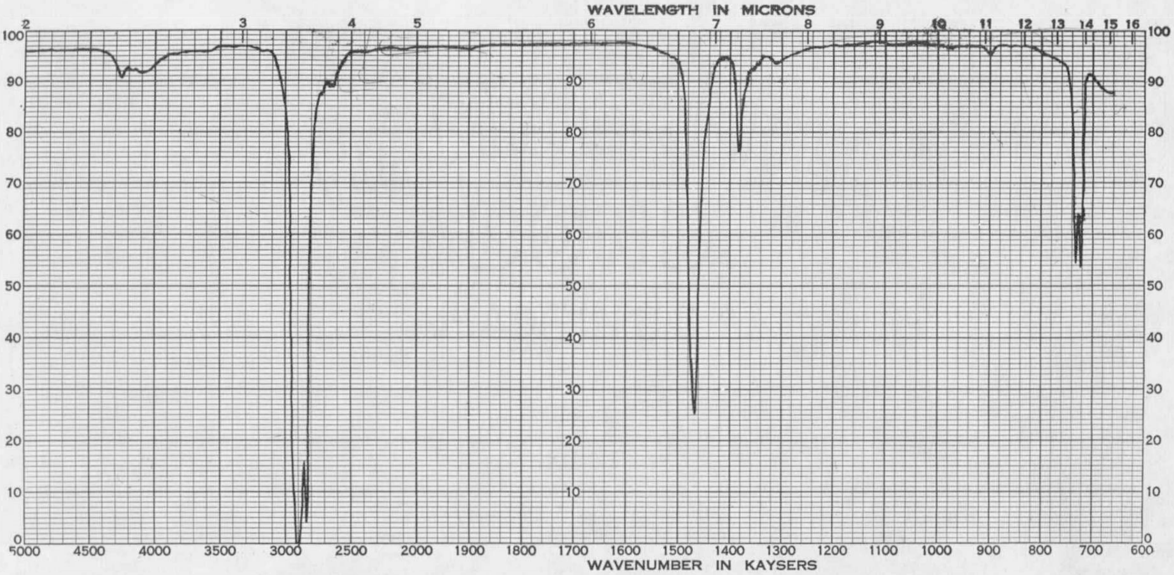


FIGURE 6. INFRARED SPECTROGRAM OF WAX K_{NH}, TUBE 5

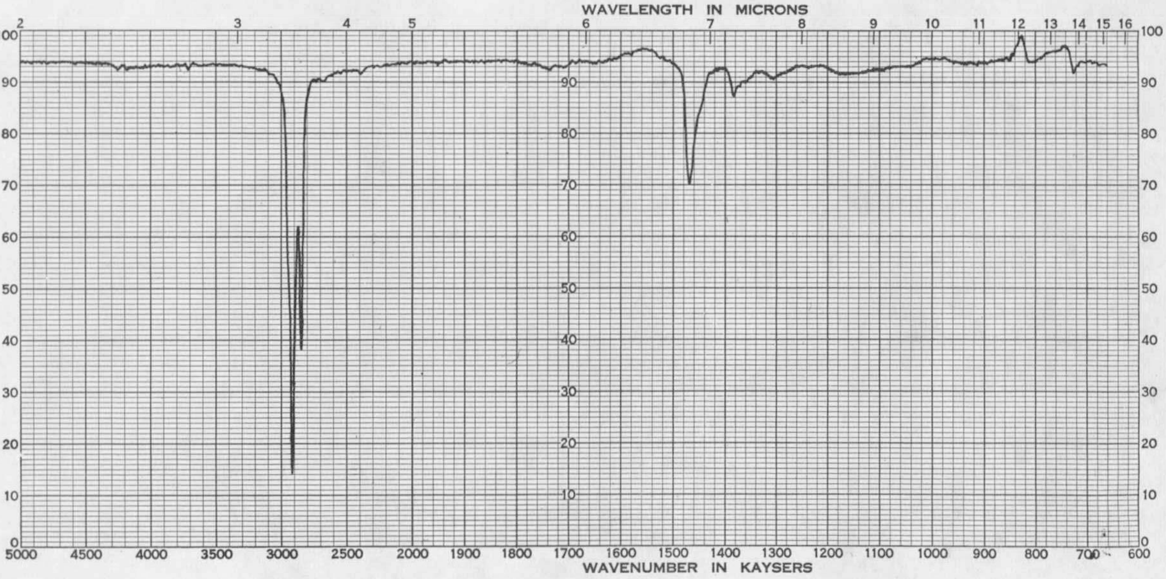
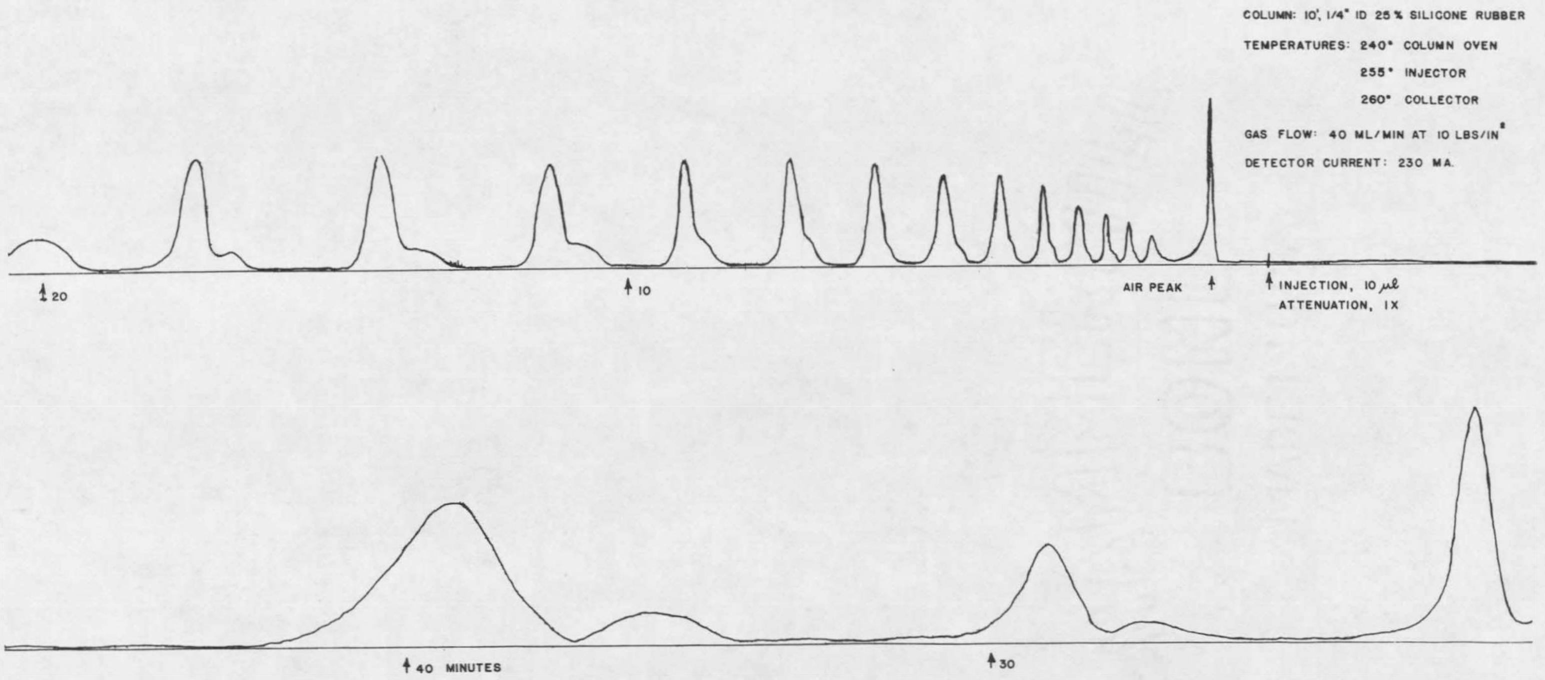


FIGURE 7. INFRARED SPECTROGRAM OF N-HEPTACOSANE

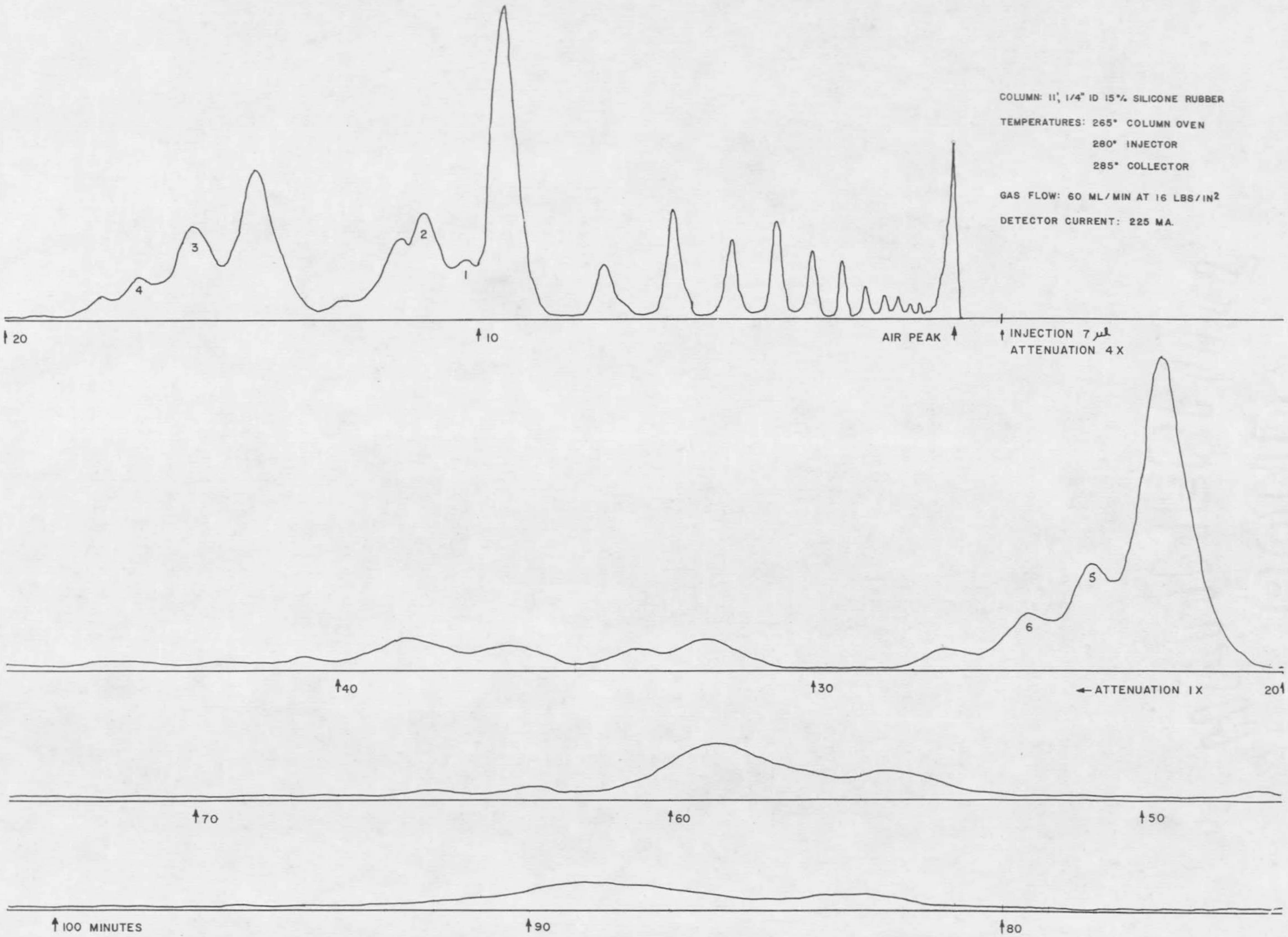
Preliminary examination of the gas chromatograms of Wax K_{NH}, Figures 8 and 9, showed evidence of several homologous series of hydrocarbons. A plot of the log of the retention time from the air peak versus the number of carbon atoms yields a straight line for a homologous series. Figures 10 and 11 are the log retention plots of the chromatograms of Figures 8 and 9, respectively. Homologous series were definitely present since straight line plots were found. In order to tie down the exact placement of the carbon numbers on the x-axis several known normal hydrocarbons were prepared and/or purchased. By a conventional Wurtz Reaction[17], using the proper bromides and metallic sodium, the following hydrocarbons were synthesized: n-dodecane (n-C_{12}), n-hexadecane (n-C_{16}), n-tetracosane (n-C_{24}), n-dotriacontane (n-C_{32}), and n-hexatriacontane (n-C_{36}). Those purchased were n-eicosane (n-C_{20}) from Distillation Products Industries and n-tetratriacontane (n-C_{34}) and n-dotetracontane (n-C_{42}) from Applied Science Laboratories. The retention times from the air peak of these compounds were compared with the unidentified peaks of Wax K_{NH}. In every case, except for n-dotetracontane, the retention time of a known was found to correspond to one of the unknowns. Virtual identification of all the normals could now be made. Wax K_{NH} was found to contain normal hydrocarbons from C_{10} through C_{36}, with C_{35} absent.

Further confirmations of the identifications of these normals followed. Paraffin wax (Standard Oil Company's "Parawax" was used) is known to contain normal hydrocarbons from C_{20} through C_{30} with some samples ranging from C_{17} through C_{34}[18]. This paraffin wax, with its components identified



COLUMN: 10', 1/4" ID 25% SILICONE RUBBER
TEMPERATURES: 240° COLUMN OVEN
255° INJECTOR
260° COLLECTOR
GAS FLOW: 40 ML/MIN AT 10 LBS/IN²
DETECTOR CURRENT: 230 MA.

FIGURE 8. LOW TEMPERATURE GAS CHROMATOGRAM OF WAX K_{NH}



COLUMN: 11', 1/4" ID 15 % SILICONE RUBBER
TEMPERATURES: 265° COLUMN OVEN
280° INJECTOR
285° COLLECTOR

GAS FLOW: 60 ML/MIN AT 16 LBS/IN^2

DETECTOR CURRENT: 225 MA.

FIGURE 9. HIGH TEMPERATURE GAS CHROMATOGRAM OF WAX K_{NH}

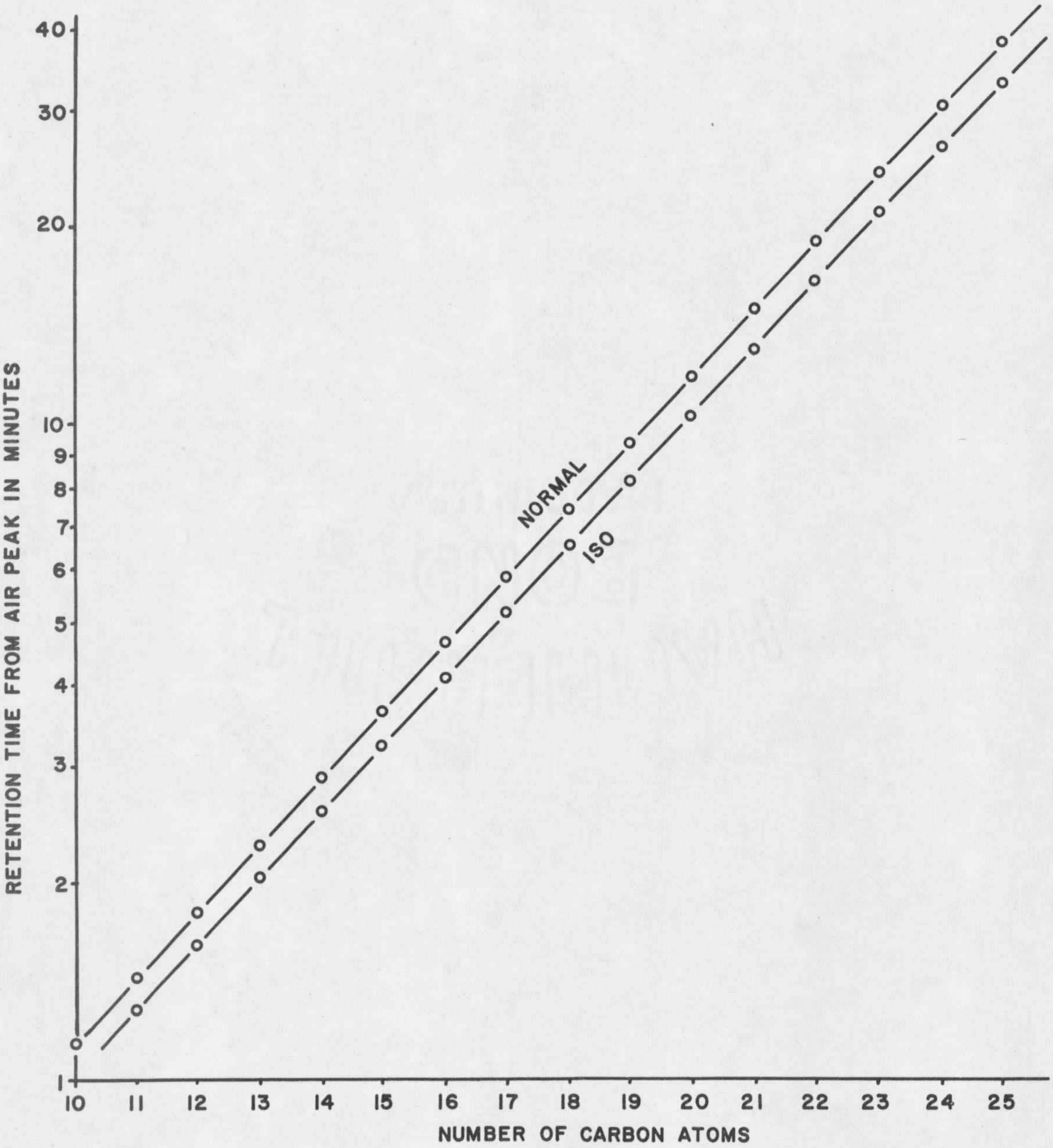


FIGURE 10. LOG OF RETENTION TIME VS NUMBER OF CARBON ATOMS OF WAX K_{NH} FROM LOW TEMPERATURE GAS CHROMATOGRAM

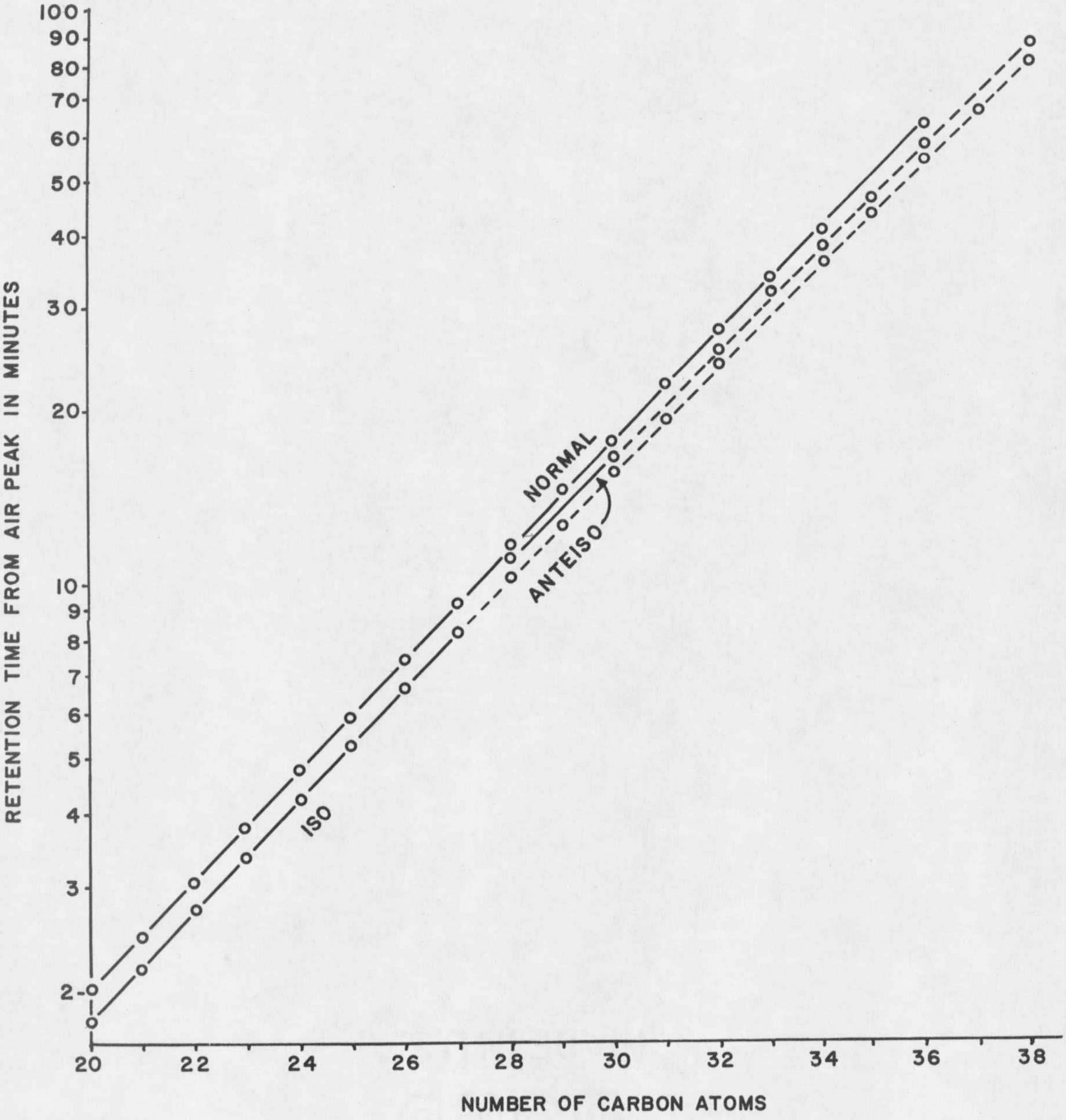


FIGURE 11. LOG OF RETENTION TIME VS NUMBER OF CARBON ATOMS OF WAX K_{NH} FROM HIGH TEMPERATURE GAS CHROMATOGRAM

by use of the knowns, provided a very good method to check the assignments of Wax K_{NH}. As before, retention times were compared and also some of the known hydrocarbons were added to the paraffin wax and the chromatogram taken. All retention times compared exactly and in no case did the added compound produce a new peak; but only enlarged one already present.

Another confirmation of the presence of the normals was given by the adsorption of these hydrocarbons by molecular sieves. Figure 12 is the gas chromatogram of Wax K_{NH} obtained with the molecular sieve pre-column at low temperature and Figure 13 is the high temperature chromatogram. Comparison of these chromatograms with those obtained without the molecular sieve pre-column (Figures 8 and 9) shows many of the peaks to be missing. These are the peaks corresponding to the normal hydrocarbons. Normal unsaturated hydrocarbons are also adsorbed by molecular sieves[15], but since none are known present in Wax K_{NH} the absence of the peaks is attributed solely to the adsorption of normal saturated hydrocarbons. The compounds that are not affected by the molecular sieves are branched and/or cyclic saturated hydrocarbons.

Some naturally occuring branched hydrocarbons are known to be of the iso structure[6, 7, 18]. Thus the hydrocarbons of the cuticular wax of the Mormon Cricket were similarly thought to contain iso hydrocarbons. On this assumption, pure samples of iso-undecane (i-C_{11}), iso-hexadecane (i-C_{16}), and iso-tetracosane (i-C_{24}) were obtained from the American Petroleum Institute. Figure 13 is a log retention plot of these hydrocarbons and some known normals. The two lines for the homologous series are quite

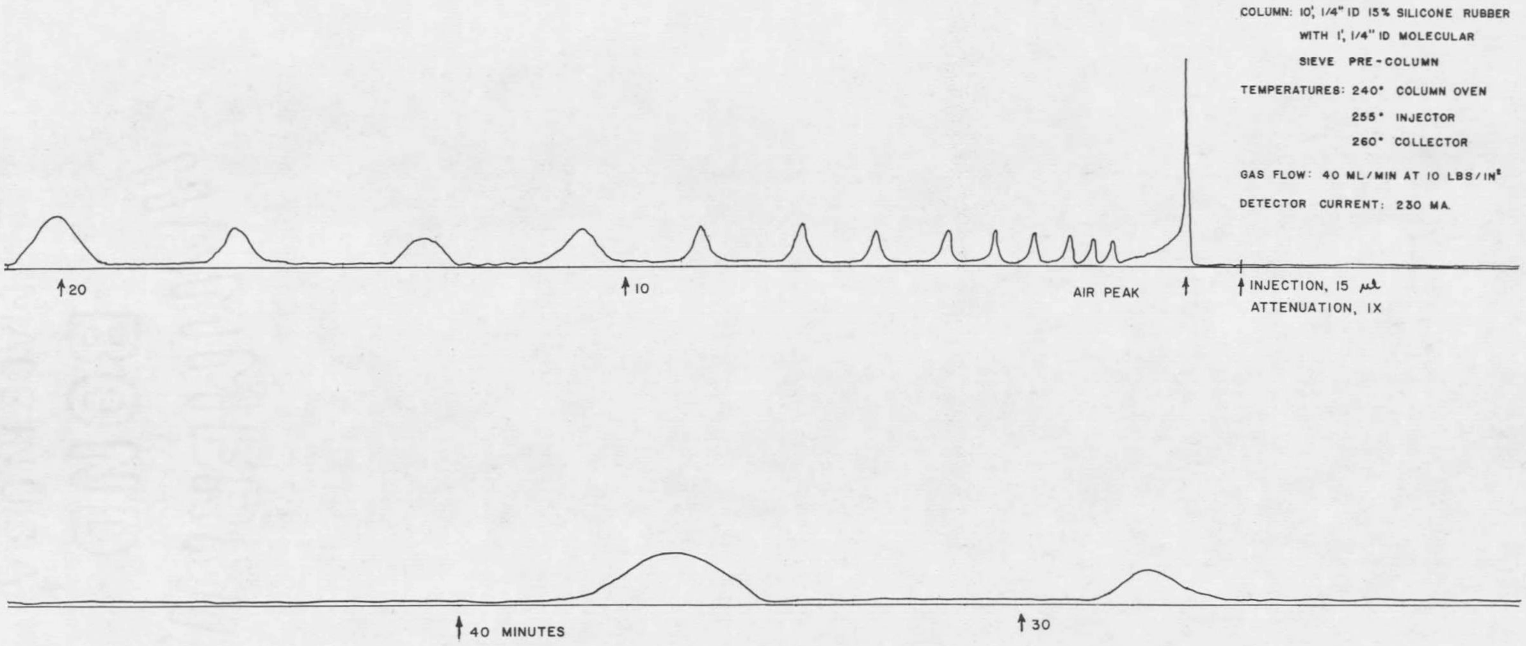


FIGURE 12. LOW TEMPERATURE GAS CHROMATOGRAM OF WAX K_{NH}

OBTAINED WITH MOLECULAR SIEVE PRE-COLUMN

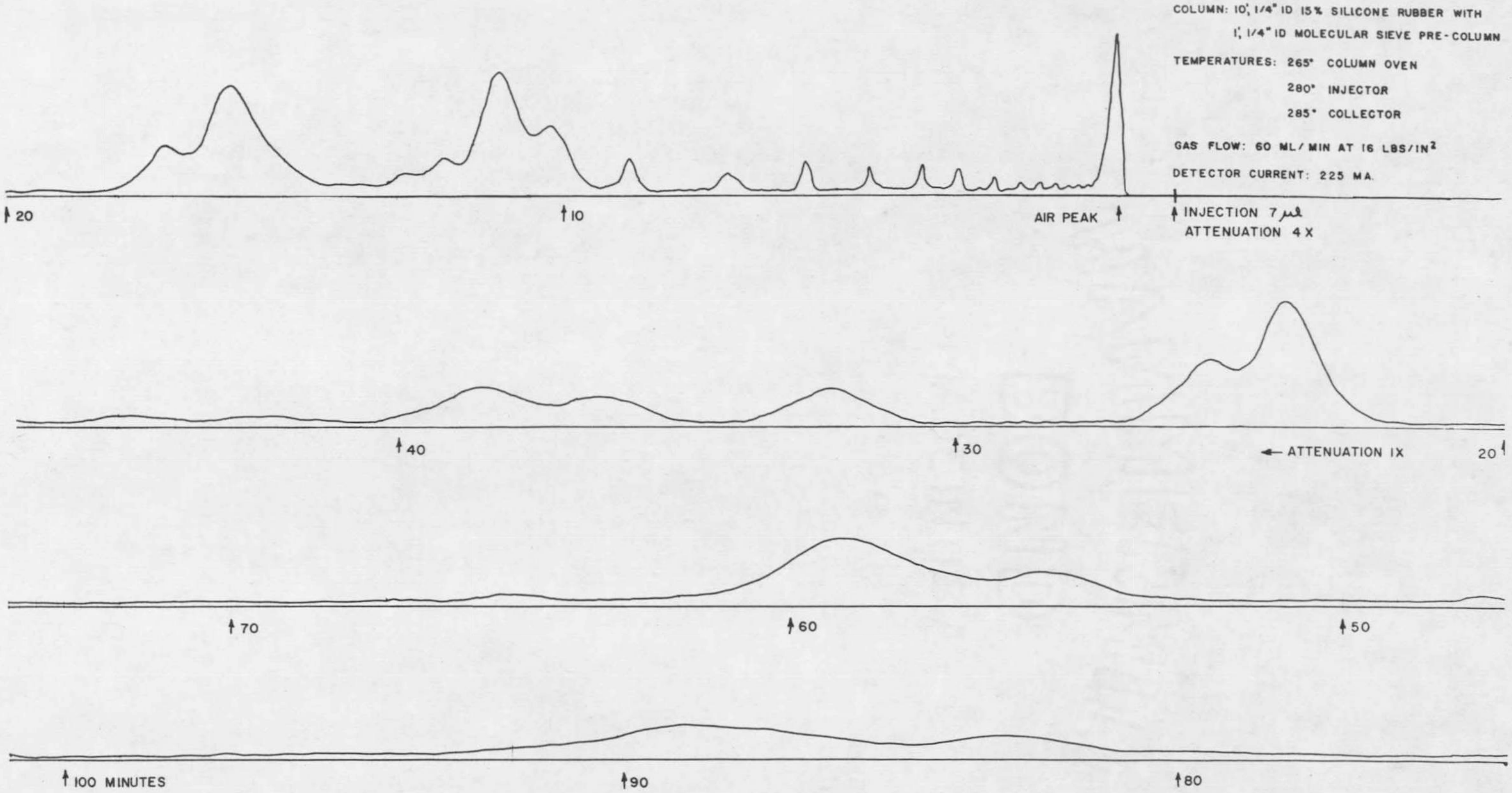


FIGURE 13. HIGH TEMPERATURE GAS CHROMATOGRAM OF WAX K_{NH}

OBTAINED WITH MOLECULAR SIEVE PRE-COLUMN

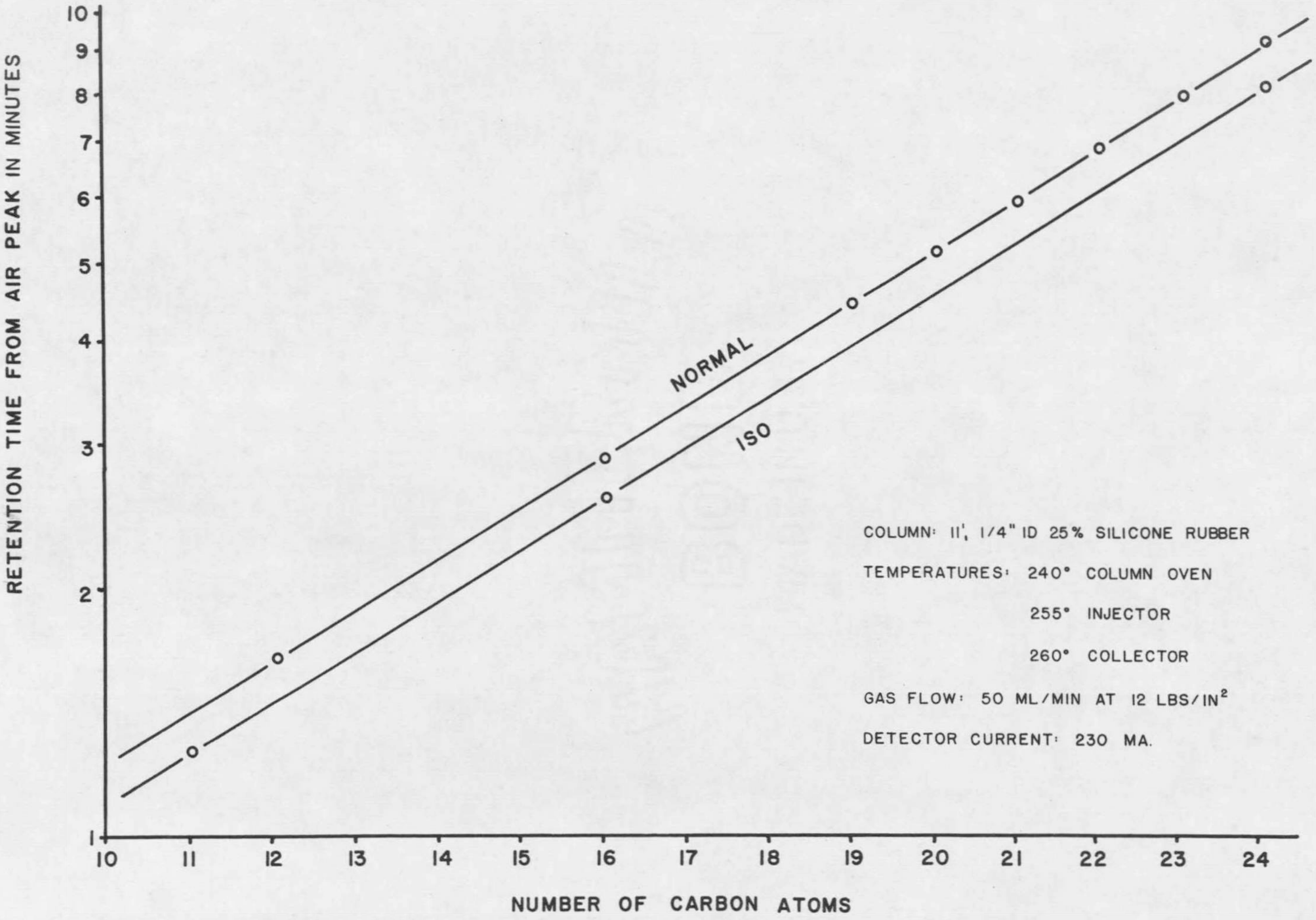
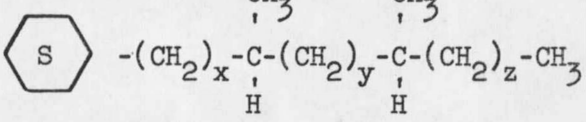


FIGURE 14. LOG OF RETENTION TIME VS NUMBER OF CARBON ATOMS OF SOME KNOWN NORMAL AND ISO HYDROCARBONS

separate and easily definable. Wax K_{NH} (see Figures 8 and 9) has what appears to be three homologous series and it seemed that in addition to the normals, one of the other series was due to iso hydrocarbons. In fact, the retention times of some iso hydrocarbons corresponded with those of some of the components of Wax K_{NH}. This appeared to indicate that a homologous series of hydrocarbons with the iso structure was present.

To further elucidate the composition of the branched-cyclic group of compounds of Wax K_{NH} several of these hydrocarbons were collected. The peaks are numbered on Figure 9. The nuclear magnetic resonance spectra of these compounds were taken by Varian Associates for the purpose of proton counting. Figure 15 is one of these spectra, specifically that of peak 3. The area under the small peak is proportional to the number of methyl hydrogens on the hydrocarbon and the area under the large peak is proportional to the number of methylene hydrogens. From the analysis of the ratios of these areas compared to the number of carbon atoms in the molecule it was determined that these hydrocarbons has only one branch. These data are shown in Figure 16. The possibility that these compounds were not mono-branched but had some other structure that would give equivalent results was investigated. It was found that compounds of the general structure



$$\text{S}-(CH_2)_x-\overset{CH_3}{\underset{H}{C}}-(CH_2)_y-\overset{CH_3}{\underset{H}{C}}-(CH_2)_z-CH_3$$

would have similar hydrogen ratios. Compounds resembling these have been found in petroleum[19] and the occurence of hydrocarbons of this nature in

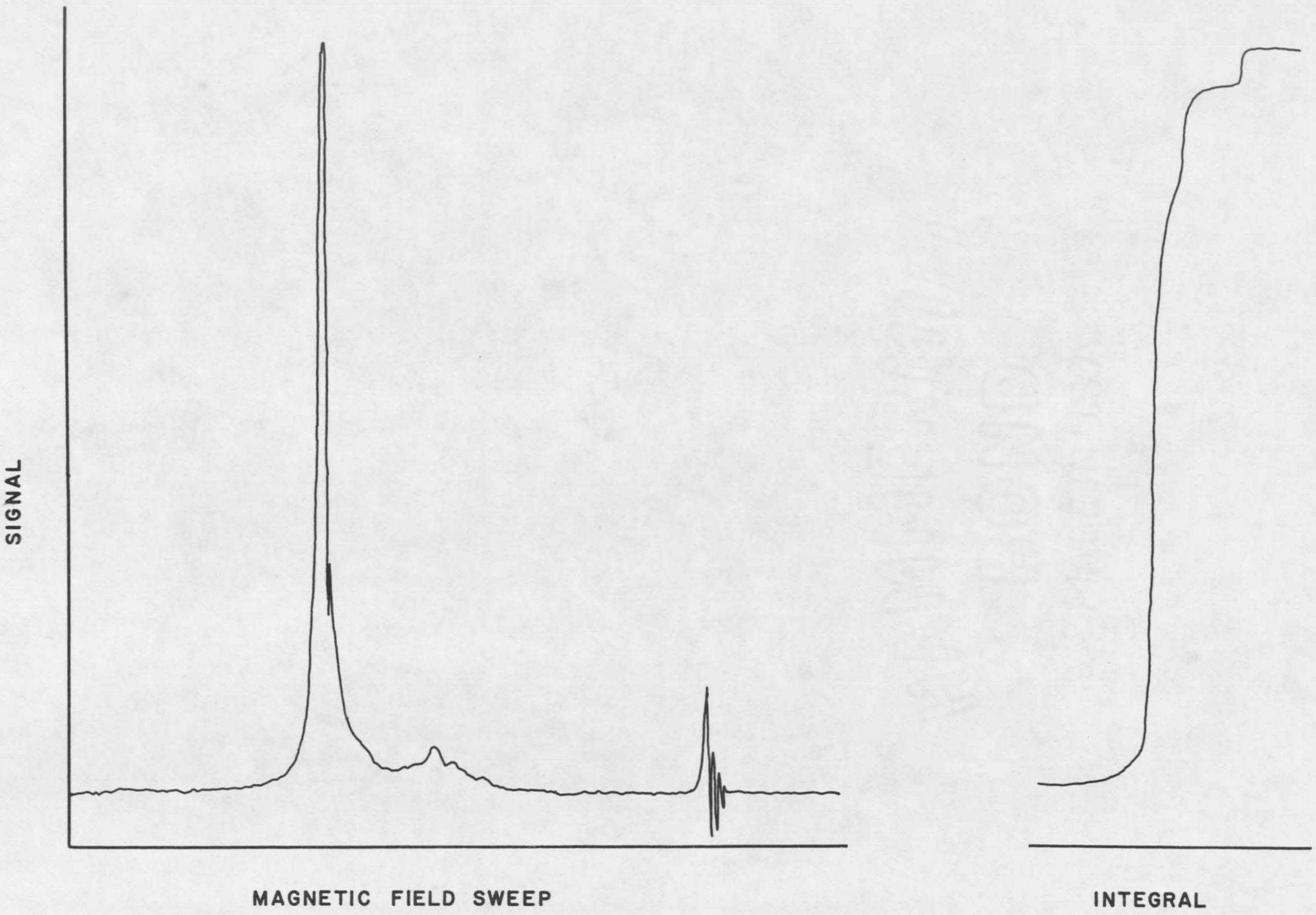


FIGURE 15. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF PEAK 3 OF WAX K_{NH}

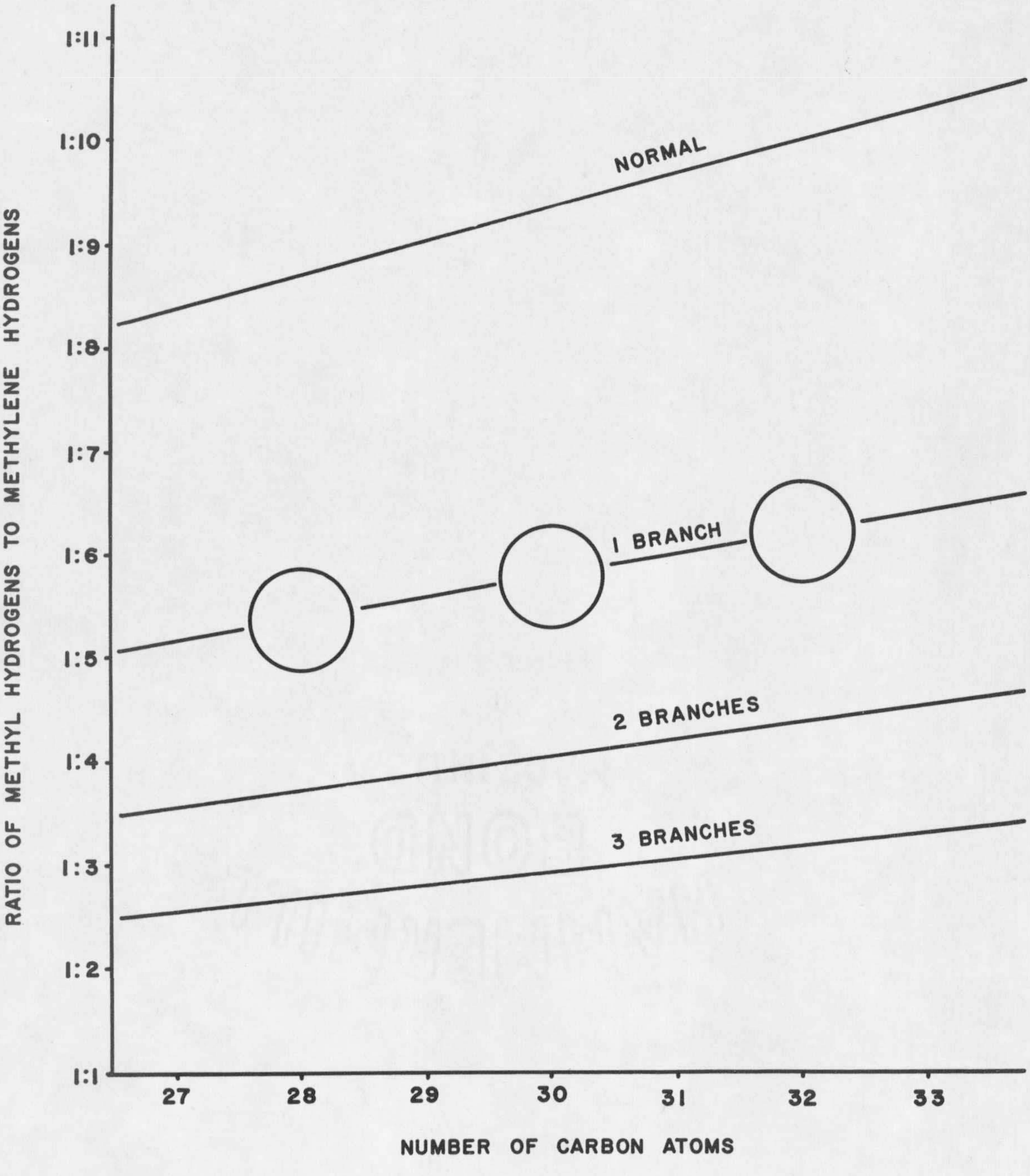
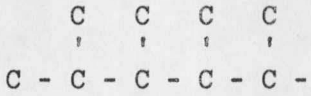


FIGURE 16. BRANCHING DETERMINATION FROM NMR DATA

the cricket wax was considered possible. To definitely locate the branch and/or determine if indeed cyclic groups were present these hydrocarbons were subjected to mass spectral analysis.

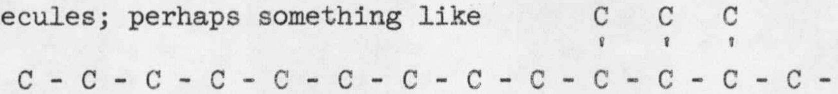
The hydrocarbons previously analyzed by N.M.R. were sent to the American Oil Company's Whiting, Indiana laboratory and their mass spectrograms obtained. The analysis of these samples from the mass spectral data is given below.

sample	composition
1.	Lost
2.	Chiefly 3-methylheptacosane. Traces of heavier paraffins are detected out to C_{37}; the sample may also contain traces of paraffins lighter than C_{28}.
3.	Chiefly C_{29} and C_{30} paraffins with many short branches; perhaps something like



$$\begin{array}{ccccc} & C & C & C & C \\ & | & | & | & | \\ C - & C - & C - & C - & C - \end{array}$$

sample	composition
4.	Chiefly 3-methylnonacosane; also some C_{29} paraffin, probably the normal. Trace of heavier paraffin gives alkyl peaks to C_{32}.
5.	Chiefly C_{31} and C_{32} with structures similar to sample 3. Trace of heavier paraffin gives alkyl peaks at C_{33} and C_{34}.
6.	Material detected as heavy as C_{34}; suspected that components extend over a range of 3 or 4 carbon numbers. The spectrum suggests multiple short branches well removed from the ends of the molecules; perhaps something like



$$\begin{array}{ccccccccccccc} & & & & & & & & & C & C & C & \\ & & & & & & & & & | & | & | & \\ C - & C - & C - & C - & C - & C - & C - & C - & C - & C - & C - & C - & C - \end{array}$$

The sample of n-heptacosane, see Figure 7, previously identified from gas chromatographic techniques was also analyzed. The mass spectral analysis of this compound not only confirmed its presence but indicated that the sample was of high purity.

The correlation between the mass spectral data and the N.M.R. data for peaks 2 and 4 was excellent. The compounds were shown to be mono-branched from their proton counts and this branch was determined to be a methyl group in the 3- position by mass spectrometry. Careful examination of Figure 11, the high temperature log retention plot of Wax K_{NH}, shows that there may be more of these anteiso (3-methyl) hydrocarbons present. Peak 6 could possibly be one of this homologous series since the N.M.R. data shows it to be mono-branched. Lack of a completely pure sample for the mass spectral study could perhaps account for the lack of total confirmation.

From gas chromatographic and N.M.R. studies, peaks 1, 3, and 5 were thought possibly to be another homologous series, probably with the iso (2-methyl) structure. The similarities of Figures 10, 11, and 14 support this conclusion since they all show one homologous series preceeding the normal series in time. Also, N.M.R. studies showed these compounds to be mono-branched. To confirm the assignment, a known sample of iso-tetracosane was added to a sample of Wax K_{NH} and the gas chromatogram taken. No new peak was observed, only the strengthening of the i-C_{24} peak. However, sample 1 was lost and the mass spectral analysis of peaks 3 and 5 indicated mixtures with multiple methyl branches. The mass spectral data and the N.M.R. data are in disagreement here. If this is a problem of incomplete separation, then only through the use of more sophisticated equipment, such as capillary columns and programmed temperature control for the gas chromatograph, can this problem be further attacked.

The method of Eastman[20] was used to determine quantitatively the amounts of the various components of Wax K_{NH}. The relation is

$$\frac{m_i}{w} = \frac{A_i \sqrt{M_i}}{\sum_i (A_i \sqrt{M_i})}$$

where m_i/w is the fraction by weight of the ith component, A_i the area under the peak corresponding to the ith component, and M_i the molecular weight of the ith component. Table I is a summary of the hydrocarbons and their amounts as determined by this relation.

RESULTS

A study of the hydrocarbons of the cuticular wax of the Mormon Cricket, Anabrus simplex Hald. has been made. An estimate of the components present and their relative amounts has been made by interpretation of this data. Of the 61 hydrocarbons known to be present, 28 have been positively identified. In terms of weight percentage, those identified constitute 75.6%. Table I is a summary of the probable hydrocarbon constituents.

TABLE I.

COMPOSITION OF THE HYDROCARBONS OF THE CUTICULAR WAX OF THE MORMON CRICKET

Type	Compound		% of Total Hydrocarbons
Normal			
	n-decane	$C_{10}H_{22}$	0.03
	n-undecane	$C_{11}H_{24}$	0.07
	n-dodecane	$C_{12}H_{26}$	0.11
	n-tridecane	$C_{13}H_{28}$	0.19
	n-tetradecane	$C_{14}H_{30}$	0.32
	n-pentadecane	$C_{15}H_{32}$	0.42
	n-hexadecane	$C_{16}H_{34}$	0.43
	n-heptadecane	$C_{17}H_{36}$	0.53
	n-octadecane	$C_{18}H_{38}$	0.60
	n-nonadecane	$C_{19}H_{40}$	0.68

TABLE I. (CONTINUED)

Type	Compound		% of Total Hydrocarbons
Normal			
	n-eicosane	$C_{20}H_{42}$	0.77
	n-heneicosane	$C_{21}H_{44}$	1.3
	n-docosane	$C_{22}H_{46}$	2.4
	n-tricosane	$C_{23}H_{48}$	3.8
	n-tetracosane	$C_{24}H_{50}$	3.3
	n-pentacosane	$C_{25}H_{52}$	6.5
	n-hexacosane	$C_{26}H_{54}$	3.4
	n-heptacosane	$C_{27}H_{56}$	18.4
	n-octacosane	$C_{28}H_{58}$	3.7
	n-nonacosane	$C_{29}H_{60}$	12.5
	n-triacontane	$C_{30}H_{62}$	1.9
	n-untriacontane	$C_{31}H_{64}$	4.0
	n-dotriacontane	$C_{32}H_{66}$	0.67
	n-tritriacontane	$C_{33}H_{68}$	0.19
	n-tetratriacontane	$C_{34}H_{70}$	0.06
	n-hexatriacontane	$C_{36}H_{74}$	0.13
Iso (Probable)			
	i-undecane	$C_{11}H_{24}$	0.01
	i-dodecane	$C_{12}H_{26}$	0.01

TABLE I. (CONTINUED)

Type	Compound		% of Total Hydrocarbons
Iso (Probable)			
	i-tridecane	$C_{13}H_{28}$	0.01
	i-tetradecane	$C_{14}H_{30}$	0.02
	i-pentadecane	$C_{15}H_{32}$	0.02
	i-hexadecane	$C_{16}H_{34}$	0.02
	i-heptadecane	$C_{17}H_{36}$	0.02
	i-octadecane	$C_{18}H_{38}$	0.03
	i-nonadecane	$C_{19}H_{40}$	0.03
	i-eicosane	$C_{20}H_{42}$	0.07
	i-heneicosane	$C_{21}H_{44}$	0.11
	i-docosane	$C_{22}H_{46}$	0.15
	i-tricosane	$C_{23}H_{48}$	0.25
	i-tetracosane	$C_{24}H_{50}$	0.31
	i-pentacosane	$C_{25}H_{52}$	0.38
	i-hexacosane	$C_{26}H_{54}$	0.33
	i-heptacosane	$C_{27}H_{56}$	0.39
Anteiso			
	a-octacosane	$C_{28}H_{58}$	6.2
	a-triacontane	$C_{30}H_{62}$	3.2

TABLE I. (CONCLUDED)

Type	Compound		% of Total Hydrocarbons
Iso (Possible)			
	i-octacosane	$C_{28}H_{58}$	3.3
	i-nonacosane	$C_{29}H_{60}$	0.29
	i-triacontane	$C_{30}H_{62}$	10.7
	i-untriacontane	$C_{31}H_{64}$	0.30
	i-dotriacontane	$C_{32}H_{66}$	1.5
	i-tetratriacontane	$C_{34}H_{70}$	0.44
	i-pentatriacontane	$C_{35}H_{72}$	0.06
	i-hexatriacontane	$C_{36}H_{74}$	0.71
	i-heptatriacontane	$C_{37}H_{76}$	0.07
	i-octatriacontane	$C_{38}H_{78}$	0.14
Anteiso (Possible)			
	a-dotriacontane	$C_{32}H_{66}$	0.49
	a-tritriacontane	$C_{33}H_{68}$	0.56
	a-tetratriacontane	$C_{34}H_{70}$	0.56
	a-pentatriacontane	$C_{35}H_{72}$	0.06
	a-hexatriacontane	$C_{36}H_{74}$	1.9
	a-octatriacontane	$C_{38}H_{78}$	1.2

DISCUSSION OF LIMITATION OF RESULTS

This discussion is included since some of the results of this study are not conclusive and some are even contradictory.

The normal hydrocarbons that were identified presented no ambiguous or contradictory results. However, there is the possibility of other normal hydrocarbons being present below C_{10} and above C_{36}. If they are indeed present, it is in amounts undetectable by the methods employed in this study.

The gas chromatographic data indicates that a homologous series of iso hydrocarbons is present from C_{11} through C_{27}. But since all of these compounds are present in such small amounts (the 17 compounds account for only 2.16% of the hydrocarbons by weight) there was no practical way to collect samples for mass spectral confirmations.

There could also be iso hydrocarbons below C_{11} and above C_{27}. The log retention plots of the gas chromatograms of Wax K_{NH} (Figures 10 and 11) indicate the possibility that the iso series may continue past C_{27} up to C_{38} with C_{33} absent. Two samples corresponding to the peaks for iso C_{28} and iso C_{30} of this extended series were collected. The N.M.R. studies were indicative of mono-branched hydrocarbons. However, mass spectrometry showed them to be mixtures of compounds containing multiple branches. This mixture could originate in at least two ways. First, due to possible insufficient resolution, a single peak on the gas chromatogram could include the peaks of two or more compounds of the original wax mixture.

Second, it is possible that isomerization may take place somewhere in the scheme of analysis. The likely place for this isomerization is in the gas chromatography. The use of platinum at temperatures above 200°C. is a commercial method to increase branching of C_6 hydrocarbons to improve octane rating. Also some commercial methods of isomerization use high surface area bauxite to support an aluminum halide catalyst. In the chromatography a copper column, filled with a diatomaceous earth support of high surface area, is heated to 300°C. Since the gas chromatographic conditions are similar to those in the commercial methods, similar catalytic action in the gas chromatography might be postulated. However, normal hydrocarbons never gave any indication of this isomerization. And the possibility of isomerization during mass spectrometry can be ruled out since the n-heptacosane was analyzed to be very pure and showed no evidence of isomerization. Thus, although isomerization cannot be completely ruled out, it does not seem likely. Therefore, the sample impurity probably originates in the wax itself and it is the failure of the gas chromatograph to resolve these individual components.

Two anteiso compounds, C_{28} and C_{30}, were found. As in the case of the iso compounds, the N.M.R. data and the log retention plots indicated the possibility of a series of anteiso compounds from C_{32} to C_{38} with C_{37} being absent. The mass spectral data of peak 6, C_{32} of this possible series, is contradictory. It suggests multiple short branches removed from the ends of the molecule. This too could be a case of impure sample either by isomerization or by having several components with nearly the same retention times.

It should be pointed out here that in no instance has the possibility of cyclic compounds been eliminated. Infrared absorption with NaCℓ optics is not very precise for determinations of this kind. A N.M.R. spectrum could show cyclic saturated hydrocarbons only if there were enough methylidyne hydrogens to give a signal greater than the instrument noise. Mass spectrometry would show identifiable cyclic groups only if not too badly masked by other acyclic components. Thus no positive identification of the possible cyclic hydrocarbons was made, but similarly no absolute denial of them is given.

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A study of the hydrocarbons of the cuticular wax of *Anabrus simplex* Hald

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