The free fatty acids and neutral lipids of the cuticular wax of the Mormon cricket, Anabrus simplex, Hald

by Joel Mackie Padmore

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Chemistry

Montana State University

Abstract:
The composition of the free fatty acids and the neutral lipids of the Mormon cricket, Anabrus simplex. Hald., was studied by infrared spectroscopy and gas, column, and thin-layer chromatography. Free fatty acids were separated on the basis of unsaturation by column chromatography of their methyl esters on silver nitrate impregnated silicic acid. The presence of normal and branched chain saturated acids (C8-C36) and unsaturated acids (C14-C24) was demonstrated by gas chromatography on SE-30 silicone arid EGS polyester columns. Structures of the major unsaturated acids, oleic, linoleic; and linolenic, were determined by infrared spectroscopy and reductive ozonolysis followed by gas chromatography. Neutral lipids were separated into chemical classes by column chromatography on various adsorbents. Wax esters, steryl. esters, triglycerides, free sterols, diglycerides, monoglycerides, and traces of residual fatty acid were found. The fatty acid components were studied by transesterification followed by gas chromatography. The free sterols and non-saponifiables derived from the ester fractions were determined by gas chromatography on SE-30 and QF-1 silicone columns.

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This thesis is dedicated to my wife, Carol, who has endured much and given her encouragement and moral support during the past four years.

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ABSTRACT

The composition of the free fatty acids and the neutral lipids of the Mormon cricket, Anabrus simplex, Hald., was studied by infrared spectroscopy and gas, column, and thin-layer chromatography. Free fatty acids were separated on the basis of unsaturation by column chromatography of their methyl esters on silver nitrate impregnated silicic acid. The presence of normal and branched chain saturated acids (C_8-C_{36}) and unsaturated acids (C_{14}-C_{24}) was demonstrated by gas chromatography on SE-30 silicone and EGS polyester columns. Structures of the major unsaturated acids, oleic, linoleic, and linolenic, were determined by infrared spectroscopy and reductive ozonolysis followed by gas chromatography. Neutral lipids were separated into chemical classes by column chromatography on various adsorbents. Wax esters, steryl esters, triglycerides, free sterols, diglycerides, monoglycerides, and traces of residual fatty acid were found. The fatty acid components were studied by transesterification followed by gas chromatography. The free sterols and non-saponifiables derived from the ester fractions were determined by gas chromatography on SE-30 and QF-1 silicone columns.

INTRODUCTION

The cuticular wax of insects has been found to be an important factor in the control of moisture balance in insects.[9,40] Abrasion of this layer frequently results in rapid dessication and death of the insect. Some insects, such as the cockroach, are restricted to moist habitats whereas other insects, such as the Mormon cricket, have the ability to survive in arid regions. A study of the nature of the cuticular lipids would lead to a greater understanding of one of the limiting factors in the geographical distribution of insects. Beament has postulated that retardation of water passage through the cuticle of the cockroach is due to the presence of a mono-layer of fatty alcohols at the lower interface of the cuticular wax.[7] In a recent study Gilby and Cox[18] showed that the lipids of the cockroach included fatty acids, aliphatic aldehydes, esters, and hydrocarbons, but no fatty alcohols; thereby partially refuting Beament's postulated mechanism of moisture control.

There have been few investigations into the chemical composition of cuticular waxes. Partial analyses have been carried out on cuticular waxes from ticks[17] and silkworm larvae.[2,32] Possibly the most complete study of an insect cuticular wax present in the literature is that of Gilby and Cox.[18] The nature of the hydrocarbons, aldehydes, and fatty acids were fairly completely elucidated. Little or no work was done to characterize the esters or to confirm the trace quantities of sterols reported.

The original investigation into the lipid composition of the cuticular wax of the Mormon cricket was initiated by J. H. Pepper and E. J. Hastings in the mid-1940's.[29] The object of the study was to learn more about the

control of water balance by studying the chemical composition of the insect cuticle. The Mormon cricket was selected because of its availability, large size, and economic importance in Montana. At that time insect waxes were generally believed to be relatively simple mixtures of long chain aliphatic alcohols, long chain aliphatic esters, and long chain aliphatic acids. The extracted cuticular wax was subjected to saponification, resulting in intractable emulsions. About 1957 the project was revived by Baker[5] who undertook a spectrophotometric and chromatographic study of the wax. This study showed the presence of esters, fatty acids, hydrocarbons, acidic resins, and possibly cholesterol. The acids and hydrocarbons were partially characterized, but no work was done on the esters and the presence of cholesterol was not confirmed.

The object of the present study has been to characterize as completely as possible the esters, free sterols, and free fatty acids. This has included the determination of double bond position and configuration of the major unsaturated acids and the identification and quantitative determination of the acids, fatty alcohols, and sterols of the various ester fractions. Methods were developed and/or modified to permit the analysis of the various fatty acids, sterols, and esters present.

EXPERIMENTAL PROCEDURE AND RESULTS

Isolation of Wax

Crickets used in this study were collected during the summers of 1961 and 1962 from various locations in southeastern Montana. The live crickets were quick frozen and stored in a freezer until excision of the cuticles. No selection was made as to sex but only adults past the fifth moult were used. The cuticles were excised under water and brushed lightly to remove adhering cellular material. The excised cuticles were dried and stored frozen until extracted.

Initial studies were performed on wax isolated by soxhlet extraction. The cuticles were held in a cup of chloroform-washed cheese cloth and extracted with chloroform for about twenty hours. The solvent was removed and the crude wax recovered.

Final studies were made on material extracted from 1962 cuticles at room temperature. Cuticles and chloroform were mixed in a large stainless steel blender and homogenized for five minutes The homogenate was allowed to stand for about ten minutes and was then filtered to remove the ground cuticles. The chloroform was reduced to about ten ml on a rotary evaporator under reduced pressure and then taken to dryness under a stream of nitrogen.

The wax isolated by both methods had similar characteristics. It was yellow-green in color, had a characteristic odor, and a softening range of 25-50 degrees. The crude wax obtained by either method was designated wax K.

Wax K was taken up in hexane and allowed to stand for about one hour. The hexane solution was filtered to remove the acidic resins and other

hexane insolubles. The filtered hexane solution was immediately passed through a Kies[22] countercurrent apparatus to remove the free fatty acids. The three stages contained 0.01N KOH, 0.005N KOH, and water respectively. Total time for an average Kies extraction was about 60 hours. Ethanol was added dropwise to the first and second stages as needed to reduce foaming.

The extracted hexane solution was taken to dryness and designated wax Kn. The infrared spectrum of Kn is shown in figure 1. The intensity of the bands at 720 and 730 cm^{-1}, the ratio of the carbon-hydrogen deformation bands at 1380 and 1470 cm^{-1}, and the intensity of the carbon-hydrogen stretching vibration near 2900 cm^{-1} indicate long hydrocarbon chains. The ester carbonyl at 1740 cm^{-1} is characteristic of that observed in triglycerides and steryl esters. The carbon-oxygen stretch near 1200 cm^{-1} is also similar in pattern to that observed for triglycerides. A small amount of fatty acid is probably still present as indicated by the shoulder at 1705 cm^{-1}, the characteristic carbonyl stretch of an aliphatic acid. Weak bands near 3300 cm^{-1} suggest the possibility of hydroxy compounds such as alcohols, sterols, monoglycerides, and diglycerides.

The combined aqueous solutions from the Kies apparatus were acidified to pH 3 with 1.0 N sulfuric acid and estracted three times with diethyl ether in 300, 300, and 200 ml portions. The combines ether extracts were washed with 25 ml of water and dried over anhydrous sodium sulfate. The ether was removed by evaporation on a rotary evaporator under reduced pressure to about 10 ml, then taken to dryness under a stream of dry nitrogen. The recovered acidic materials were designated was Ka. The infrared

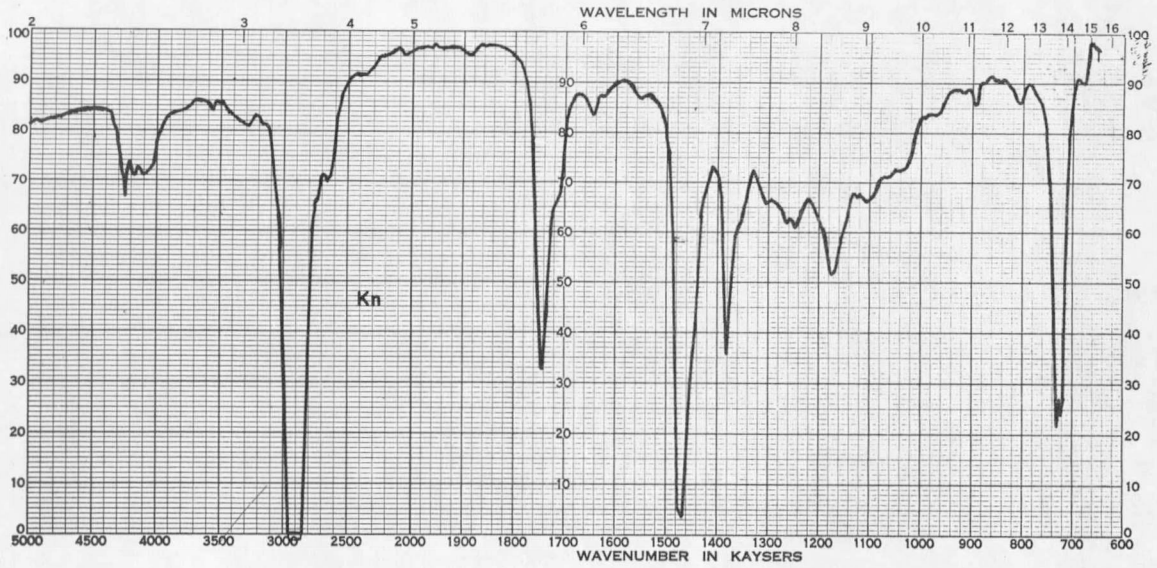

Figure 1. Infrared spectrum of Wax Kn (cap. film.)

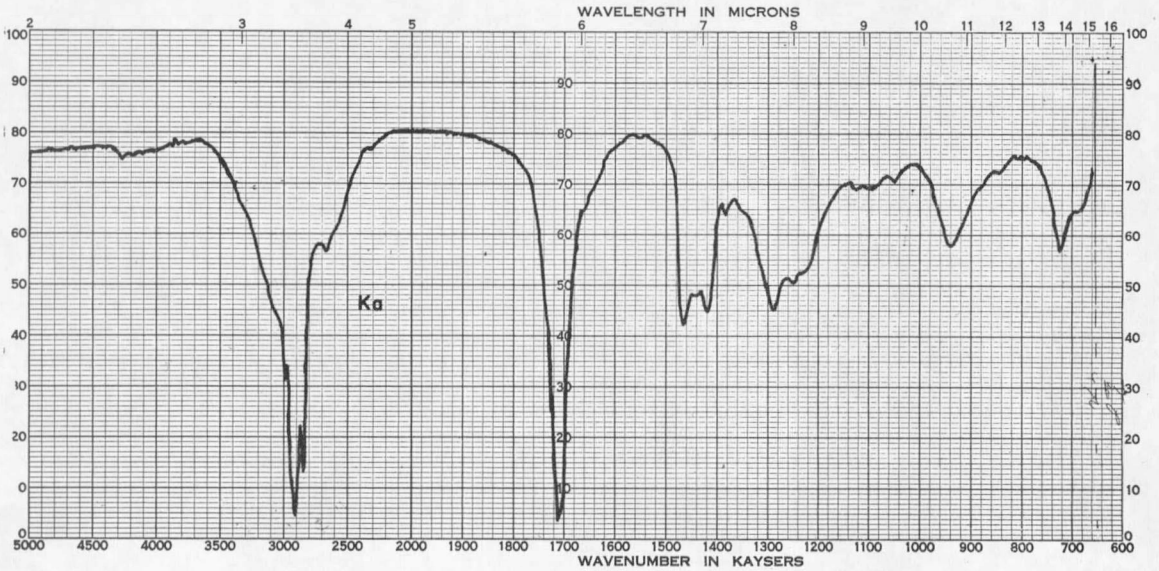

Figure 2. Infrared spectrum of Wax Ka (cap. film.)

spectrum, figure 2, is typical of spectra obtained from long chain fatty acids. The weak band just below 3000 cm^{-1} on the side of the carbon-hydrogen stretching vibration indicates the presence of unsaturation. Table I shows the relative amounts of hexane insolubles, neutrals, and acids obtained.

TABLE I

Composition of Wax K

Fraction	Weight Percent
hexane insolubles	1.3
Wax Ka	31.3
Wax Kn	67.4

Infrared Spectra

All infrared spectra were obtained on a Beckman IR-4 Spectrophotometer equipped with sodium chloride optics with presentation linear in wave-numbers. Spectra were taken as capillary films whenever possible. Spectra of solids were obtained in both carbon disulfide and carbon tetrachloride.

Column Chromatography

All chromatography columns, regardless of adsorbent used, were prepared in an identical manner. The weighed adsorbent was slurried with hexane and placed into a 1.2 cm column. The column was lightly tapped to insure uniform packing of the adsorbent; the adsorbent was covered with a filter paper