A STUDY OF THE LIPID FRACTIONS IN THE POLLEN OF ZEA MAYS

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ABSTRACT

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IN THE POLLEN OF *ZEA MAYS*

by Amir Fathipour

A considerable number of papers have been published on the chemical constituents of pollens. Analyses have been made largely for minerals, amino acids, pigments, carbohydrates, vitamins and lipids. In the study of the latter substances many of the fatty acids and lipids were not isolated and characterized due to the lack of proper methods. However, the development of gas-liquid chromatography and improved techniques in column and thin-layer chromatography have greatly facilitated the isolation and identification of lipids. The methods previously mentioned, were employed to study the lipids in the pollen of *Zea mays*.

Complete extraction of the lipid material was accomplished by extracting the corn pollen with a boiling mixture of chloroform-isopropanol (1:1) v/v solution. After extraction, the solution was evaporated to a small volume *in vacuo* and the concentrated extract was diluted with chloroform and water. The chloroform layer was removed, washed with water and evaporated to dryness. Further resolution was made by dissolving the residue in chloroform and separating

the extract by column chromatography into phosphatides and non phosphatides. Fractionation of the nonphosphatides by thin-layer chromatography yielded sterol esters, triglycerides, free fatty acids, free sterols, methyl esters of fatty acids and other glycerides. Identification of the individual fatty acids in each fraction and their concentration were obtained after transmethylation by gas-liquid chromatography. Lauric, myristic, myristoleic, arachidic and behenic acids were characterized for the first time. Other acids, also identified and previously reported, were palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids.

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By

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INTRODUCTION

Pollen plays an important role in the life cycle of plants and has interested scientists for many years to learn more about the physiology and biochemistry of pollen as it relates to fertilization. The importance of pollen in the production of food products has aroused many physiologists and became one of the first of the natural products to be investigated.

Horticulturist and plant breeders have cross-bred many varieties, species and genera to produce new and improved types of plants better suited for food. Entomologists have been interested in pollen since it is the chief source of all nutrients required by bees. Tsitin (48) claimed that the longevity of most of the centenarians in Russia depended on their diet of honey.

In recent years pollen research has been influenced largely by the fact that it is responsible for certain allergies. Consequently, much of the recent literature is devoted to the studies of these allergies.

Analysis of a large number of different pollen for specific materials have been reported, but only thirteen pollens have had relatively complete analysis (49). The fatty acid content of the pollen of Zea mays was studied by Barr et al. (4) but the analyses were incomplete because

modern techniques were unavailable. The development of thin-layer and gas-liquid chromatography and the improved techniques in column chromatography have enabled scientists to determine and fractionate the lipids and their fatty acids in biological materials with a considerable degree of accuracy. This present work is an extension of the work by Barr *et al.* (4) for the determination of the lipids and associated fatty acids in the *Zea Mays* of pollen.

HISTORICAL

Storage and Viability

Systematic studies of the storage of pollen were started in 1886 by Mangin (26). Johri and Vasil (17), reviewed the literature on the effect of temperature, relative humidity, gases, and pressures on the viability of stored pollen. They concluded that low temperatures and a relative humidity of 25 to 35 percent are necessary to retain viability for a prolonged period. The viability of stored corn pollen has been reviewed by Andronescu (2).

Culture

The early history of pollen tube physiology was reviewed in 1961 by Johri and Vasil (17) who described the effects of pH, sugars, hormones, vitamins, carotenoids, antibiotics, gibberellic acid and inorganic salts on the culture of pollen. These substances are known to improve germination in many cases.

Boron is the most effective inorganic salt. The effect of boron on pollen germination and on elongation of tubes is more marked than that of any known hormone, vitamin or chemical. It increases absorption of sugars, aids in their metabolism by forming a sugar-borate complex, increases the

oxygen uptake, and is involved in the synthesis of pectic materials required for the wall of actively elongating pollen tubes. Pollen of most species seems to be naturally deficient in boron.

Chemistry of the Pollen

In comparison with the number of studies on the storage and culture of pollen, very little work has been done on the chemistry of this substance. The major limitation in this field is the difficulty in obtaining sufficient quantities of uniform samples. Consequently, studies have been restricted to plants producing relatively large amounts of pollen: e.g. Pinus and Zea mays. Nevertheless, the chemistry of pollen is gaining attention, partly, because of its importance in physiological studies and also due to its nutritional and medicinal value. Lunden (24), and Johri and Vasil (17) have written short review articles on the chemistry and amino acids of pollen.

The protein content of pollen is of interest to medical workers as an allergenic factor and to beekeepers as a source of food for better honey production. Chromatographic techniques have provided very helpful quantitative and qualitative methods for the determination of amino acids. These analytical techniques indicate that the content of amino acids of pollen proteins varies from species to species and may be as high as 35%, as reported by Todd and

Bretherick (49). Almost all the common amino acids were from pollen grains, either in the free state or bound to protein. The presence of phenylalanine, tryptophan, hydroxyproline, tyrosine and aminobutyric acid is not very common, but a high proline content is reported by Bellartz (6) and Bathurst (5). Weaver and Kuiken (51) studied the amino acid composition of pollen protein and that of soybean flour, casein and whole egg powder. They did not show any marked differences. The histidine-proline quotient in pollen (50) probably represents an important factor in pollen fertility. Ray Sarkar et al. (39) determined the amino acid composition of sweet corn pollen.

The proteins of pollen have fairly low molecular weights (5000). Some are combined with sugar. Free-proteins and those combined with sugars or pigments (artefolin, trifidin, and pratensin) have been isolated and purified. Nucleoproteins have also been reported, however, more work appears necessary (24).

Stanley (45) recorded an interesting observation concerning deoxyribonucleic acid turnover in pine pollen. Deoxyribonucleic acid is known to be synthesized when cells are ready to divide; however, Stanley presents data to confirm the presence of deoxyribonucleic acid in vegetative nuclei of pollen nuclei which do not divide.

Nutritive Value of Pollen

Pollen grains appear to be highly nutritive materials as far as water soluble vitamins are concerned. The pollens of gymnosperms have a low vitamin content, whereas, those of angiosperms are rich in vitamins of the B complex but poor in fat-soluble vitamins (36). Biotin, folic acid, inositol, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, thiamine, ascorbic acid, and vitamins A, D, E, and K have been reported in the pollen of maize, date, plum and many other plants (42).

Folic Acid has been reported in the pollen of Zea mays and many other plants by Nielsen and Homstrom (34). This acid appears to be a very important component of the so-called Vitamin T_1, a growth factor for insects. Kakhidze and Medvedyeva (18) observed that the pollen of tobacco excretes considerable quantities of vitamins into artificial germinating media. Nielsen (35) indicated that while most of the vitamins from the pollen of several species including Zea mays did not show any appreciable change after a year of storage (in a cool, dry place) the panthothenic acid content had substantially decreased in all of the samples.

Hormones and Related Substances

Estrone and estrogenic substances have been reported in the pollen of Salix and date palm by Lunden (24) and also

by Hassan and Aboul Wafa (15). Anderson (1) observed a mixture of phytosterols in corn pollen.

Mitchell and Whitehead (30), Wittwer (52), and Redemann (40), reported growth promoting substances from corn pollen which gave a positive Avena response. Others have reported similar substances from many other pollens. Fukui et al. (13) have reported 3-indoleacetic acid and two other unidentified growth promoting substances from the acidic fraction of ether extract of corn pollen.

Tanaka (46) believes that the slow growth of the pollen tube of Pinus densiflora may be due to the presence of inhibitors in its own pollen.

Enzymes

Pollen tubes metabolize externally supplied sugars and digest the food reserve while passing through the tissues of the style which indicate that they secrete enzymes (37). As expected, the presence of many enzymes and coenzymes have been reported from the pollen of different sources including maize (24, 14, 40, 34).

Carbohydrates and Lipids

Miyake (31, 32) determined the carbohydrate content of corn pollen. Nielsen et al. (36) evaluated the reducing sugars from the pollen of several species including Zea mays. Mameli (25) reports that generally anemophilous plants

(wind pollinated) have starchy pollen. Those of entomophilous plants are rich in fat and sugar which serve as a nutrient for insects, but this hypothesis has not yet been completely confirmed. Heyl (16) showed the presence of formic, acetic, valeric, a C_{10} unsaturated, myristic, palmitic and oleic acids in corn pollen. A high content of heptacosane is indicated in sugar beet pollen (20). In hazel pollen, a C_{12} acid, palmitic acid, tricosane, hexadecanol, and two unsaturated sterols have been found (44). Tappi and Monzani (47) isolated and identified heptacosane, sitosterol and phytofluene from the pollen of Lilum candidum. Mariella et al. (28) have separated seven different compounds from the unsaponifiable fraction of ragweed pollen. Barr et al. (4) obtained the following fatty acids from the saponifiable fraction of the ether extract of corn pollen: palmitic, stearic, oleic, linoleic and linolenic. Phytosterol palmitate, a saturated hydrocarbon, a saturated alcohol and lecithin have been shown to be present in corn pollen (1, 31).

Pigments

Most of the pigments are located in the holes of pollen in the form of oil and fats. These pigments serve as a filter to protect the germinating power of the pollen from ultraviolet light. The pollens of wind pollinated flowers rarely contain carotenoids. Redemann (40) reported quercetin in the pollen of Zea mays.

EXPERIMENTAL

Materials

Pollen was obtained by surrounding the tassels of sweet corn with paper bags and collecting the pollen from them after two days. The fresh pollen was screened through a 100-mesh sieve and immediately stored in plastic bags at -20°C.

Extraction of Lipids

Several methods have been described for extracting lipids from biological materials (10). A standard method of fat extraction with ethyl ether is described (3), but in the case of corn pollen complete extraction of lipid was not accomplished with this procedure, as was shown by a quantitative determination of the extract. Anderson (1) used chloroform and ethanol to extract lipids from corn pollen and he reported 13.88% total fat obtained from corn pollen (dry weight basis). Barr et al. (4) using diethyl ether as the extracting solvent reported only about 4% as the amount of fat present in corn pollen. This contradictory result indicated that diethyl ether does not extract the lipid completely from the tissues. Although tissue extract obtained by using chloroform-methanol as the

extracting solvent is known to give complete extraction of lipid materials (7), prolonged standing of tissue in methanol is known to cause transmethylation because of the presence of sodium carbonate in the tissue (23). Diethyl ether being a solvent of low polarity does not permeate the tissue sufficiently to carry polar lipids through the cell membrane quantitatively. The same is true of chloroform or petroleum ether as extractants. Extraction with chloroform-isopropanol gives quantitatively the same amount of extract as chloroform-methanol as shown by gas-liquid chromatographic analysis. The use of isopropanol eliminates the possibility of transmethylation. Table 1 summarizes the data obtained by different extraction methods.

The extraction procedure finally adopted was as follows: twenty gm. of lyophillized polled was extracted with 200 ml. boiling isopropanol (freshly redistilled) for three minutes on a steam-bath. The suspension was filtered through a fat-free filter paper and the pollen re-extracted twice in the same manner (19). The pollen residue was extracted with boiling chloroform-isopropanol (1:1) v/v. three times. Each time the solution was filtered through a fat-free filter paper and the pollen re-extracted. The final residue on the filter paper was then washed with 200 ml. of chloroform.* The combined filtrates were concentrated

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*Chloroform was redistilled in a 6 ft. Stedman Column, washed with one-half its volume of distilled water 5 times in a separatory funnel, dried over $CaCl_2$ over-night, and redistilled over P_2O_5 (41).

TABLE 1

COMPARISON OF VARIOUS METHODS OF EXTRACTION OF LIPIDS FROM CORN POLLEN

Solvent	Extraction Method	Weight of Extract (e) g.	Weight of Extract %
Carbontetra-chloride	Soxhlet 30 hrs.	0.802	1.61
Chloroform (Alcohol-Free)	Soxhlet 30 hrs.	2.015	4.03
Ethyl Ether (Alcohol-Free)	Soxhlet 30 hrs.	1.912	3.82
Ethyl Ether (2% Alcohol)	Macerated twice in 24 hrs.	2.179	4.36
Chloroform-Methanol (2:1)	Maceration (a) and Soxhlet	7.108	13.85 (b)
Chloroform-Isopropanol	(c)	6.820	13.64 (d)

(a) Method of Bottcher *et al.* (7)

(b) Weight percent after washing according to Folch *et al.* (12).

(c) Extracted according to Kates and Eberhardt (19).

(d) After being washed according to Kates and Eberhardt (19).

(e) From 50 g. of pollen.

under vacuum in a flash evaporator to 40 ml., diluted with 200 ml. of chloroform and added dropwise in a separatory funnel to 200 ml. of water. The chloroform layer was separated and the water layer was washed four times with 50 ml. of chloroform. The combined chloroform layer was evaporated to dryness under vacuo in a flash evaporator. The residue was dissolved in 20 ml. of chloroform (each ml. equivalent to 1 g. original material).

Column Chromatography Fractionation

Preparation of Silicic Acid-Celite Mixture

200 gm. silicic acid (Mallinckrodt) and 50 g. celite (100–mesh) (John Manville Corporation) were mixed and washed in a Buchner funnel with 100 ml. of chloroform, then with 1000 ml. of methanol, and finally with 1000 ml. of distilled water (9). The silicic acid-celite mixture was dried and heated at 110-120° C. for four hrs. (22) and then stored in a vacuum desiccator.

Column Packing

A slurry was made by adding 75 g. of silicic acid-celite mixture to 100 ml. of chloroform. A 2.8 cm. (i.d.) glass column fitted with a Kimax stopcock and sintered glass disk was used. The slurry was poured slowly into the column by means of a long stem glass funnel with constant stirring by use of an electric motor connected to a long glass rod. The column was left to settle for one hr. and

was washed with 200 ml. of chloroform at the rate of 2 ml/min. (under low nitrogen pressure) with proper manipulation of the stopcock.

Application of the Sample

Ten ml. of the chloroform solution of the pollen extract was applied to the top of the adsorbent in the column by means of a long stem funnel (avoiding contamination of the side of the column). After the sample was adsorbed into packing, the side of the column was washed with 20 ml. of chloroform. Then the adsorbent was eluted 18 times the column volume with chloroform (1800 ml.) The adsorbent was further washed with 50 ml. of chloroform and the latter fraction concentrated to 0.5 ml. The completeness of the elution of nonphosphatides was checked in this fraction by means of thin-layer chromatography and the Liebermann-Burchard reaction for sterols. The result of the separation of the lipid material into two fractions by column chromatography is given in Table 2.

Thin-Layer Chromatography and Fractionation of Nonphosphatides

Preparation of Thin-layer Chromatography Plates

Thin-layer plates having a dimension of 20 x 20 cm. were employed in this experiment. Before analysis the plates were scoured with Ajax detergent under running warm water, soaked in cleaning solution over night, rinsed under

TABLE 2

FRACTIONATION BY COLUMN CHROMATOGRAPHY OF THE LIPID EXTRACT OF CORN POLLEN

Fraction #	Elution Solvent	Volume of Eluent Collected ml.	Weight mg.	Total Extract %
1	Chloroform	1800	395	28.95
2	Methanol	1000	908	66.56
		TOTAL		95.51

tap water and distilled water and finally dried in the oven (38).

Thirty-two g. of silica-gel G was added to 65 ml. of distilled water to make a slurry and then shaken for 30 sec. The plates were coated to a thickness of 250-300 microns with an applicator, dried in air, and then activated in a preheated oven at 110° C. for 20 min. prior to use (33).

Application of the Sample

The sample, 138.25 mg. of lipid in chloroform, was applied as streaks to the plates. Figure 1 indicates the separation of different classes of lipid extract obtained by different extraction methods from corn pollen.

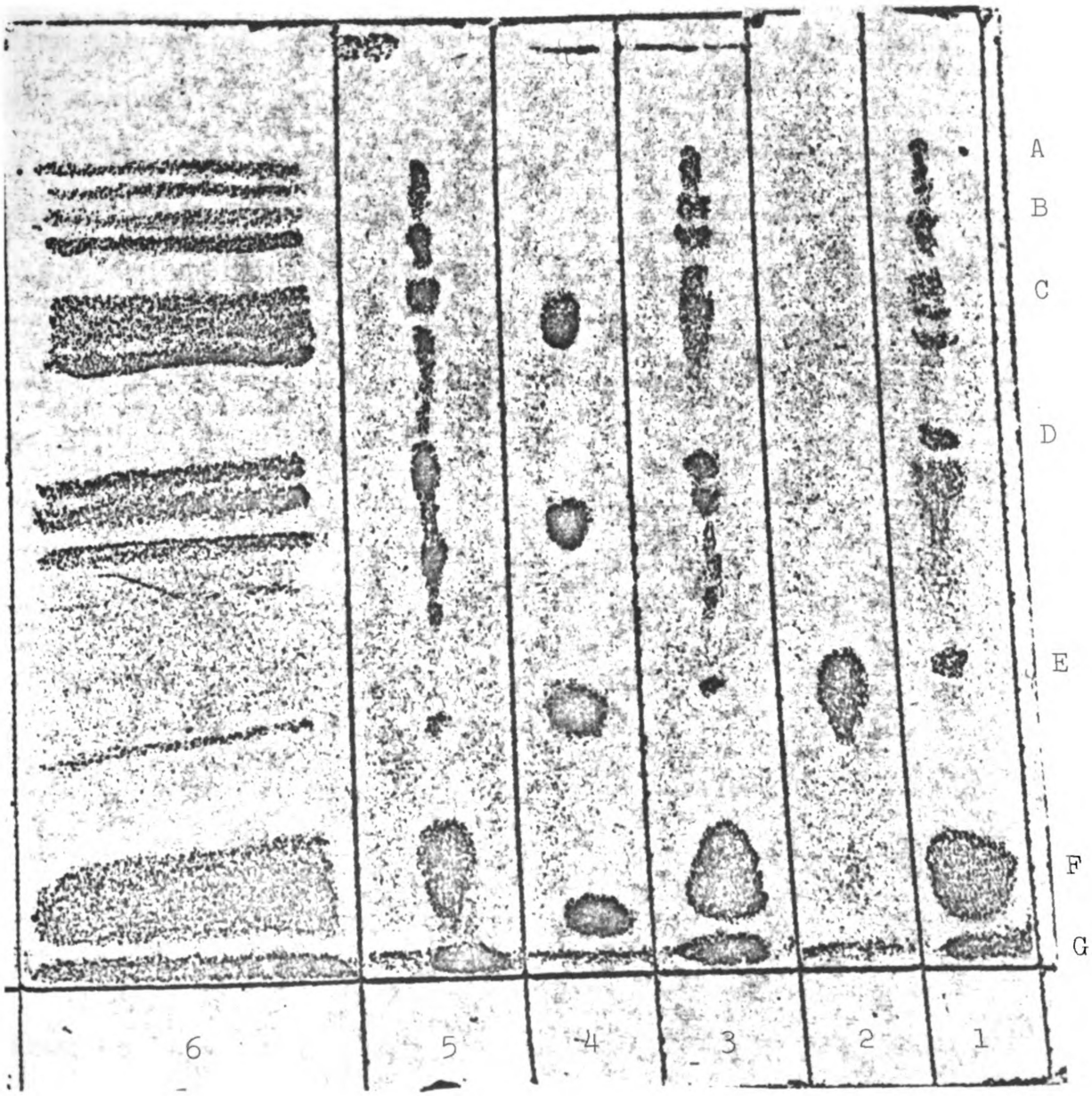
FIGURE 1

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF DIFFERENT CLASSES OF NONPHOSPHATIDES ON SILICA GEL G

The sample was resolved in a solvent system of petroleum ether-ethyl ether-acetic acid (84:16:1 v/v) for 65 min. for a distance of 15 cm.

The columns represent from left to right: extract from chloroform-isopropanol applied as streaks; extract from carbon tetrachloride; standards; extract from chloroform-isopropanol; purified fatty acid methyl esters; and extract from chloroform-methanol.

The standards (third column from left) are, from bottom to top, sterol esters, methyl esters of fatty acids, triglycerides, and free fatty acids.


A
B
C
D
E
F
G
6
5
4
3
2
1

Chromatograms were developed in freshly redistilled light petroleum ether (40-60° C.), ethyl ether* and acetic acid. The ratio of the mixture differed according to the compounds to be separated. For resolution of sterol esters from the methyl ester of fatty acids, ethylene-dichloride was used as the developing solvent (38).

Separation of different classes of nonphospholipids was accomplished by streaking 100 ml. of extract on each plate and developing the plate in petroleum ether (40-60° C.) diethyl ether and acetic acid (90:10:1) (27). The plates were air dried for 15 minutes and sprayed with 0.2% solution of 2'7'-dichloro-fluoresceine in 95% ethanol. The spots were observed under short wave length U.V. light (27).

Under the above conditions the separation of the fatty acid methyl esters and the sterol esters were not completely resolved. The methyl esters after being scraped off the plates were highly contaminated with the sterol esters and further fractionation was necessary.

Purification of Methyl Esters by Thin-layer Chromatography

For further purification of methyl ester fraction from the sterol esters, the adsorbent was washed with chloroform on a Buchner funnel, the filtrate was evaporated

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*To avoid formation of any ester in the presence of methanol and/or ethanol in ethyl ether, ethyl ether (Mallinkrodt) was washed in a separatory funnel with 1/3 of its volume of a 10% solution of NaCl (21), dried over $CaCl_2$ overnight, and redistilled.

under vacuo to a very small volume, and rechromatographed by thin-layer chromatography using ethylenedichloride as the developing solvent. Streaks showing corresponding R_f values to known methyl esters were scraped off and extracted with chloroform and filtered on a Buchner funnel. The filtrate was taken to dryness and adjusted to a known volume before chromatographed on a gas-liquid chromatograph for analysis of its fatty acids content. The various acids are listed in Table 3 and are also represented on the chromatogram, Figure 2.

Sterol Esters

The combined adsorbent containing the sterol esters was removed from the plates and transferred directly to a 50 ml. round bottom flask. Methylation was accomplished by addition of 15 ml. of 10% solution of concentrated sulfuric acid in dry methanol, and refluxing for one hr. in the presence of hydroquinone (8). After methylation the mixture was transferred to a separatory funnel with 15 ml. of distilled water and the methyl esters were extracted twice with 30 ml. of light petroleum ether (40-60° C.)

The combined petroleum ether layers were washed with 30 ml. of water, dried over sodium sulfate-sodium bicarbonate (4:1), and the solvent evaporated. The methyl esters obtained in this manner were further purified by column chromatography (19) before being studied on gas-liquid chromatography.

TABLE 3

DISTRIBUTION OF THE METHYL ESTERS OF FATTY ACIDS IN CORN POLLEN

Methyl Esters	µg./g.	%
Laurate	2.21	0.86
Myristate	5.12	2.01
Palmitate	162.62	63.82
Stearate	2.55	1.00
Oleate	5.20	2.04
Linoleate	9.25	3.63
Linolenate	65.47	25.69
Archidate	2.27	0.89
Behenate	0.20	0.07
TOTAL	254.80	100.01

Purification of Methyl Esters by Column Chromatography

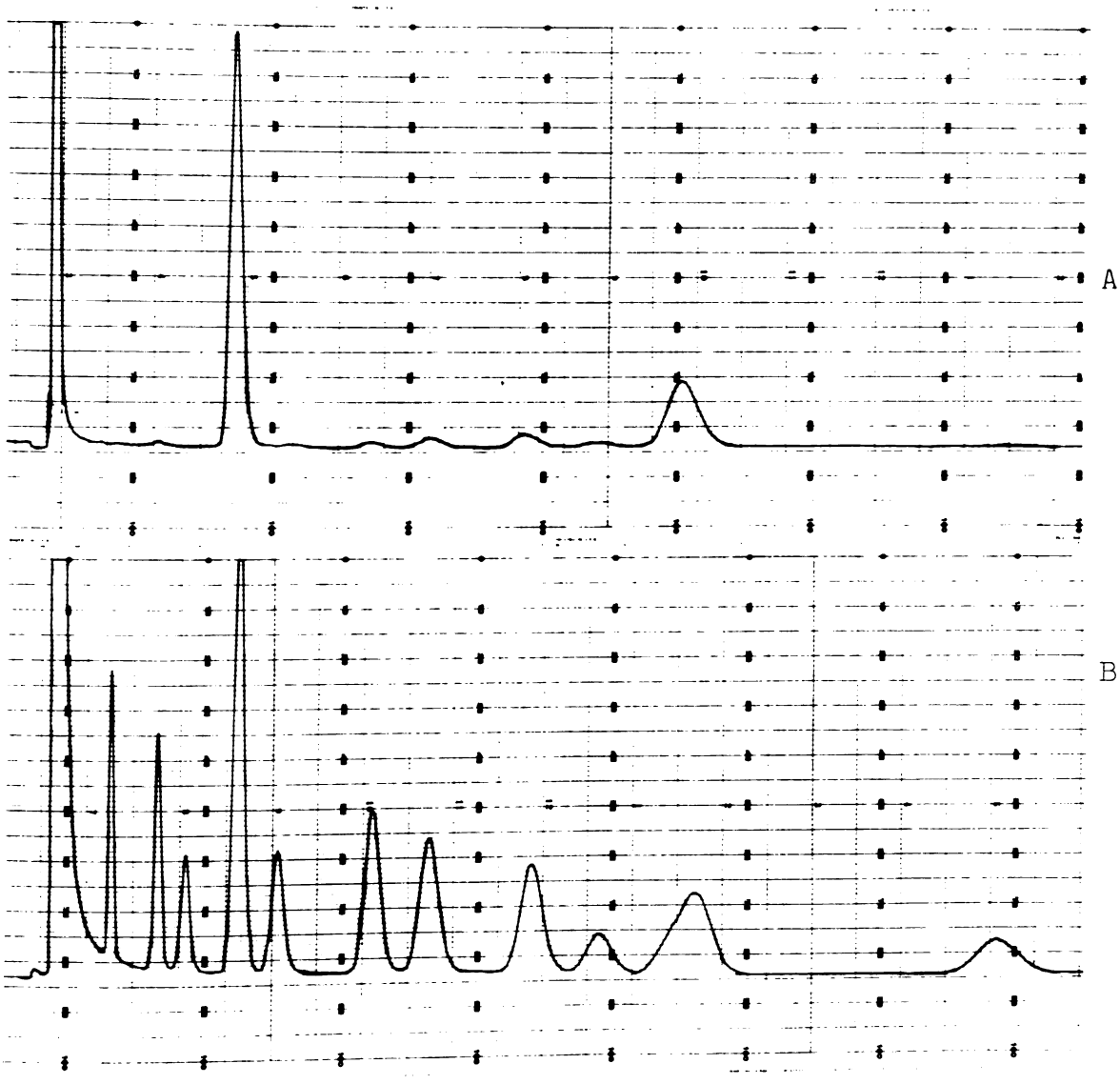
Preparation of the Adsorbent

Eight g. of silicic acid and 2 g. of celite were mixed and activated at 110-120° C. for four hrs., then transferred to a crystallizing dish, and placed in a desiccator containing 4 ml. of water. The adsorbent after being equilibrated overnight was transferred to a sealed bottle (19).

FIGURE 2

GAS-LIQUID CHROMATOGRAPHY OF NATURALLY OCCURRING FATTY ACID METHYL ESTERS

A, represent the sample and B, standards. The peaks in the standards are, from left to right, methyl esters of lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, cis-eicos-5-enoic, linolenic, and behenic acids.


A
B

Column Chromatography

A slurry was prepared by mixing 10 g. of silicic acid-celite (4:1) and 30 ml. of light petroleum ether (40-60° C.). The contents were poured into a 2 cm. (i.d.) column and washed with 100 ml. of petroleum ether. The sample of impure methyl esters was placed on the top of the adsorbent in the column and the column packing was subsequently eluted with 250 ml. of petroleum ether. The eluate was concentrated to 2 ml. and subjected to gas-liquid chromatography. The results are given in Table 4 and Figure 3.

TABLE 4

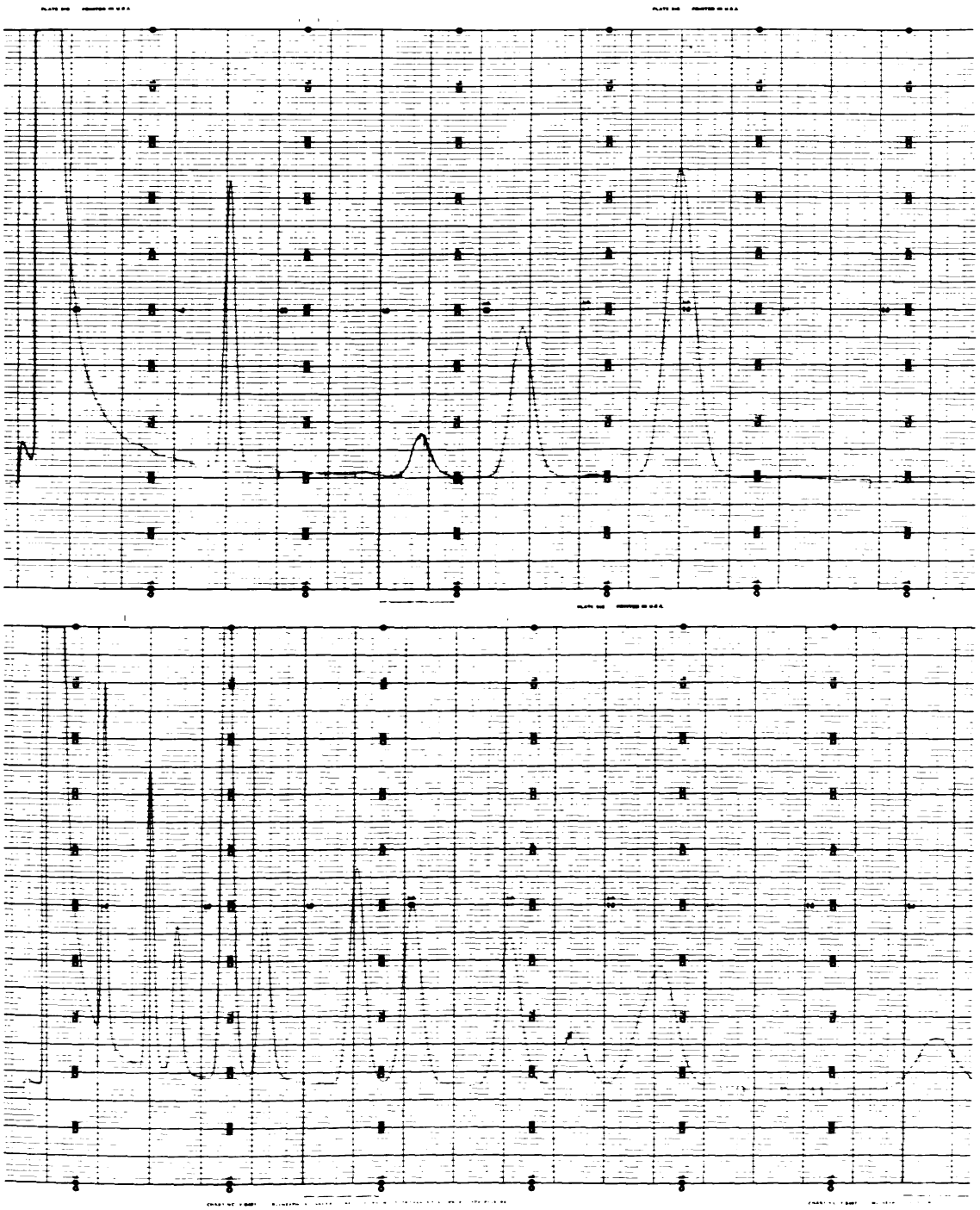
DISTRIBUTION OF THE METHYLATED FATTY ACIDS FROM THE STEROL ESTERS IN CORN POLLEN

Methyl Esters	µg./g.	%
Palmitate	555.5	19.06
Stearate	Trace	---
Oleate	125.0	4.29
Linoleate	546.8	18.74
Linolenate	1686.0	57.84
Arachidate	Trace	
TOTAL	2913.2	99.93

FIGURE 3

GAS-LIQUID CHROMATOGRAPHY OF FATTY ACIDS FROM THE STEROL ESTERS

A, represent sample and B, standards. The peaks in the standard represent, from left to right, methyl esters of lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, eis-eicos-5-enoic, linolenic, and behenic acids.



A

B

Separation of Triglycerides

Triglycerides separated in the same manner as mentioned for sterol esters were contaminated with free fatty acids, and further purification was necessary.

The solvent system which gave the best resolution of triglycerides and free fatty acids was petroleum ether, ethyl ether and acetic acid (82:18:1) (43). The triglyceride fraction obtained from first thin-layer chromatogram was re-chromatographed in this solvent system. The triglycerides separated in this manner were directly trans-methylated in the presence of hydroquinone (8), as mentioned before, and then the methyl esters were extracted and purified before being analyzed with the use of the gas-liquid chromatograph. The fatty acids present in the triglyceride fraction are given in Table 5.

TABLE 5

DISTRIBUTION OF THE METHYLATED FATTY ACIDS FROM THE TRIGLYCERIDES IN CORN POLLEN

Methyl Esters	µg./g. of Pollen	%
Myristate	5.85	0.58
Palmitate	862.96	86.05
Stearate	22.55	2.24
Oleate	23.52	2.34
Linoleate	23.30	2.32
Linolenate	48.71	4.85
Arachidate	15.91	1.58
TOTAL	1002.80	99.96

Free Fatty Acids

The free fatty acid fraction was esterfied in dry methanol with 10% H_2SO_4 at 60° C. for one hr. in the presence of the adsorbent. A crystal of hydroquinone was added to avoid oxidation. The methyl esters were extracted twice with 30 ml. of petroleum ether after addition of water. The combined petroleum ether extract was washed with water, dried over a mixture of anhydrous sodium sulfate-sodium bicarbonate (4:1), taken to dryness under vacuum adjusted to a known volume, and injected in gas-liquid chromatography column. The results shown in Table 6 and Figure 4.

TABLE 6

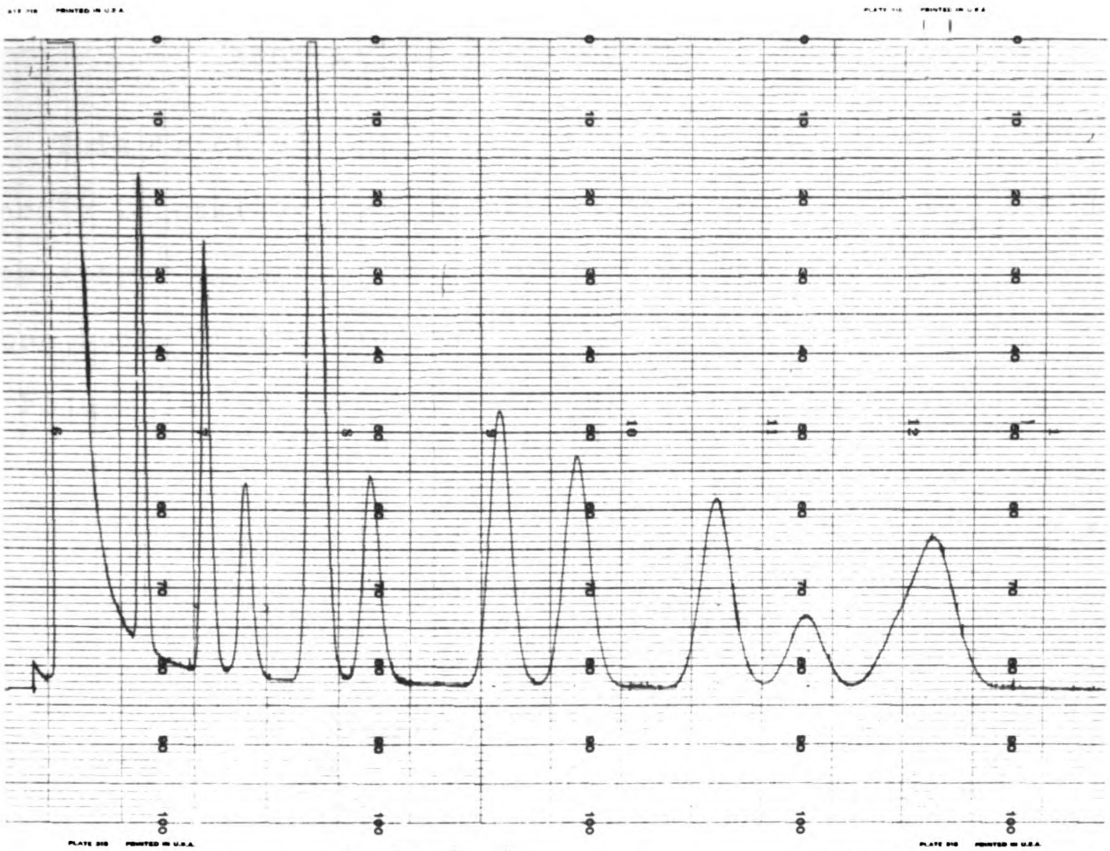
DISTRIBUTION OF THE METHYLATED FREE FATTY ACIDS IN CORN POLLEN

Methyl Esters	µg./g.	%
Laurate	2.48	.03
Myristate	27.04	.40
Myristoleate	4.48	.06
Palmitate	5988.31	91.59
Palmitoleate	45.41	.70
Stearate	116.36	1.80
Oleate	116.86	1.81
Linoleate	28.26	.43
Linolenate	93.55	1.45
Arachidate	115.91	1.79
Behenate	Trace	
TOTAL	6537.66	100.06

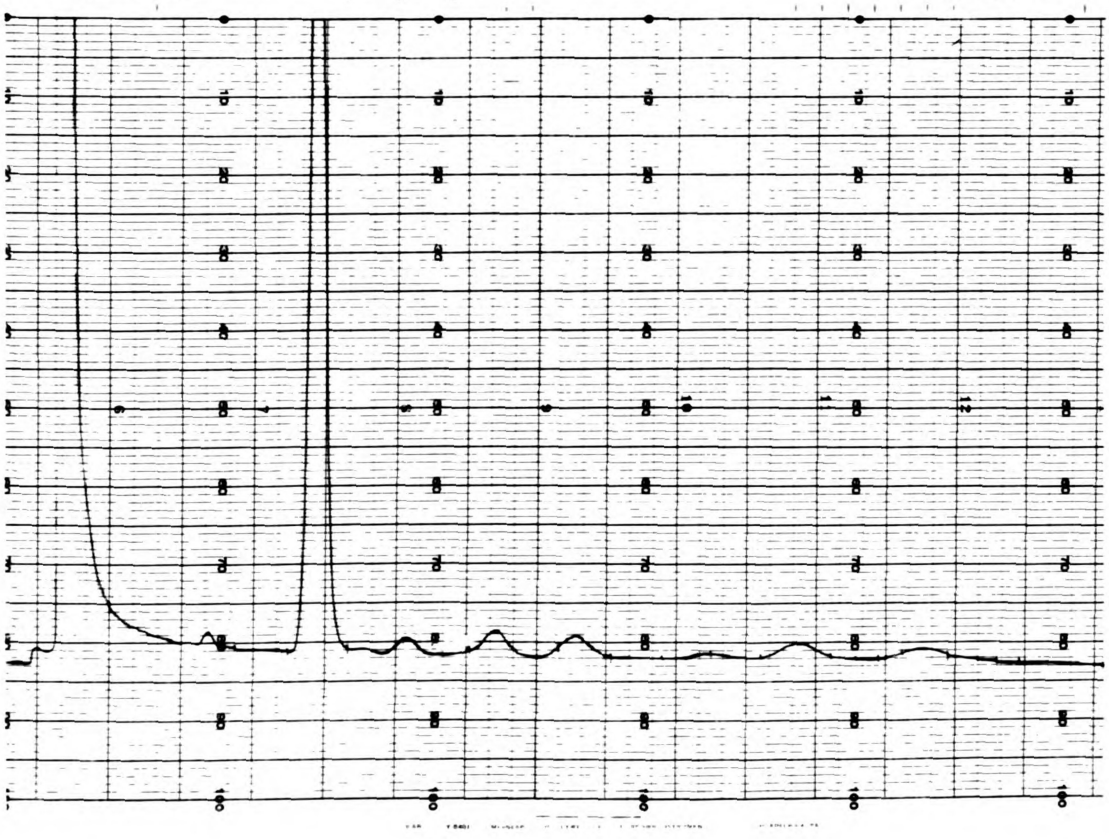
FIGURE 4

GAS-LIQUID CHROMATOGRAPHY OF METHYL ESTERS FROM THE FREE FATTY ACIDS

A, represents the standards and B, sample. The standard represent, from left to right, methyl esters of lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, cis-eicos-5-enoic and linolenic acids.



A



B

Mono-and/or Diglycerides

This fraction, which was mixed with free sterols, was directly transmethylated under the same conditions mentioned under sterol esters. The methyl esters were purified (19) before being analyzed by gas-liquid chromatography. Table 7 and Figure 5A indicate the results.

TABLE 7

DISTRIBUTION OF METHYLATED FATTY ACIDS FROM THE MONO- AND/OR DIGLYCERIDES IN CORN POLLEN

Methyl Esters	µg./g.	%
Myristate	13.60	0.28
Palmitate	3620.00	63.25
Stearate	58.46	1.02
Oleate	156.09	2.72
Linoleate	181.72	3.17
Linolenate	1634.63	28.56
Arachidate	44.84	0.78
Behenate	13.60	0.23
TOTAL	5722.94	99.96

FIGURE 5

GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF THE FATTY ACIDS FROM THE MONO- AND/OR DIGLYCERIDES AND THE PHOSPHOLIPIDS

A, represents the fatty acids from the mono- and/or diglycerides and B, standards; and C, the fatty acids from the phospholipids. The peaks of the standards from left to right represent the methyl esters of lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidic acids.

Phospholipids

The separation of the phospholipids from the total lipid extract was achieved by the use of column chromatography. Fraction 2 of Table 3 represents the fraction with phospholipids. These phospholipids were not further separated and were transmethylated as the other lipids with sulfuric acid and dry methanol. The methyl esters were analyzed by gas-liquid chromatography and the associated acids are listed in Table 8 and Figure 5C shows the chromatogram.

TABLE 8

DISTRIBUTION OF METHYLATED FATTY ACIDS FROM THE PHOSPHOLIPIDS IN CORN POLLEN

Methyl Esters	µg./g.	%
Myristate	11.53	0.11
Palmitate	8809.25	85.56
Stearate	123.75	1.20
Oleate	191.85	1.86
Linoleate	143.33	1.39
Linolenate	922.43	8.95
Arachidate	93.05	0.90
TOTAL	10295.19	99.97

Total Fatty Acids

One g. of pollen was extracted with chloroform-isopropanol and the combined extract was evaporated to dryness *in vacuo*. The methyl esters were prepared by refluxing the extract in dry methanol with 10% sulfuric acid and a crystal of hydroquinone for 16 hrs. The methyl esters were then extracted twice with 30 ml. petroleum ether, dried over sodium sulfate-sodium bicarbonate, and evaporated *in vacuo*. Purification of the methyl esters by column chromatography was done before analysis by the gas-liquid chromatograph. The results of the analysis of the fatty acids are summarized in Table 9 and Figure 6.

TABLE 9

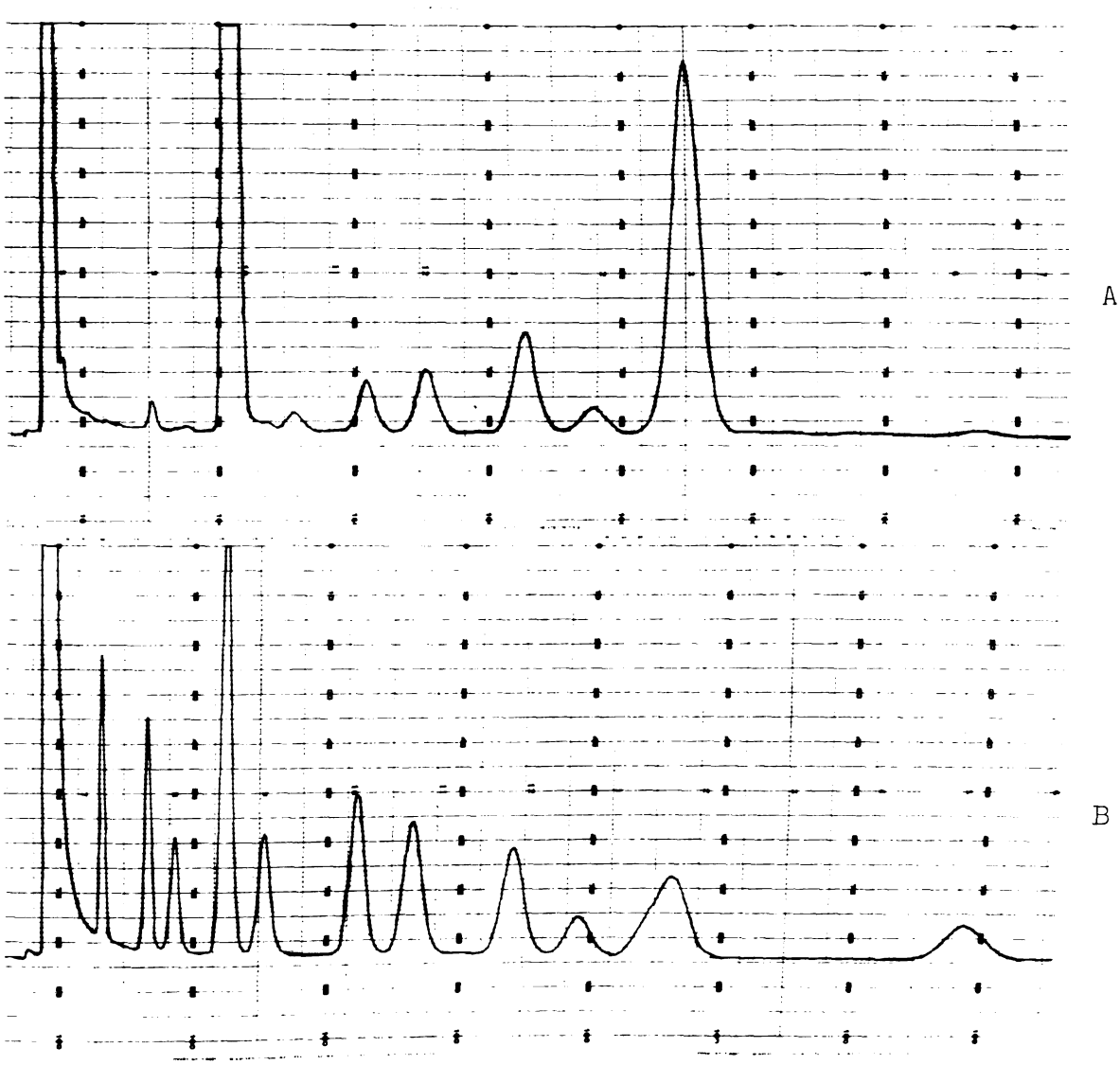
DISTRIBUTION OF THE TOTAL METHYLATED FATTY ACIDS IN CORN POLLEN

Methyl Esters	µg./g.	%
Laurate	Trace	
Myristate	53.8	0.19
Myristoleate	16.9	0.06
Palmitate	20789.0	75.03
Palmitoleate	94.5	0.34
Stearate	364.2	1.31
Oleate	660.0	2.38
Linoleate	720.0	2.59
Linolenate	4585.0	16.54
Arachidate	378.0	1.36
Behenate	44.0	0.16
TOTAL	27,705.4	

FIGURE 6

GAS-LIQUID CHROMATOGRAPHIC ANALYSIS
OF THE TOTAL FATTY ACIDS

A, represent the sample and B, standards. The peaks in the standards, from left to right, represent methyl esters of lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic linoleic, arachidic, linolenic and behenic acids.


A
B

Gas-Liquid Chromatography

The gas-liquid chromatography was accomplished on a F & M 400 "Biomedical Gas-liquid Chromatograph" with hydrogen flame ionization detector. Gas-liquid chromatography packing was done according to Desty (11) and Miller (29) on a 1/4 inch (i.d.) x 6 foot glass column with 15% diethylene glycolsuccinate polyester on diatoport S, 80-100 mesh* and conditioned overnight at 210° C. The conditions for chromatography were as follows: oven temperature, 195° C. flash heater 235° C.; sensitivity--attenuation 32 and range 10; and the flow of carrier gas (helium) 45 ml./min. Injection of the sample was done with a microliter syringe.

Thin-layer chromatography and gas-liquid chromatography standards were obtained from Applied Science Laboratories.** The area under the peak was calculated by multiplying 1/2 of the peak height by the perpendicular midway between the peak and the base. The concentration of the sample was determined by comparing the peak areas of the sample with that of peak areas with known concentration.

Results and Discussions

Sweet corn pollen was found to contain 13.64% lipids from a chloroform-isopropanol extract. On fractionation by

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*Applied Science Laboratory, State College, Pennsylvania 39-B LAC 3R-728 (Polyester).

**Applied Science Laboratories, State College, Pennsylvania.

column chromatography the following two fractions were obtained: neutral lipids, 3.95%, and acidic lipids, 9.68%. The neutral lipids were further fractionated by thin-layer chromatography and the following classes of lipids were identified: sterol esters, methyl esters of fatty acids, triglycerides, free fatty acids, free sterols, and other glycerides.

Each fraction was transmethylated and the derived fatty acid methyl ester was identified by gas-liquid chromatography. The fatty acids of sterol esters comprised 10.5% of the total fatty acids. These were linolenic, palmitic, linoleic and oleic acid in decreasing order of concentration. Six and one-half percent of the total fatty acids were in the triglyceride form. Most of the triglycerides occur as tripalmitin and trilinolenin. The other glycerides consist of 20.65% of the total fatty acids. Twenty three and one-half percent of all fatty acids are in the free state in corn pollen. Palmitic acid was the most abundant acid in this fraction.

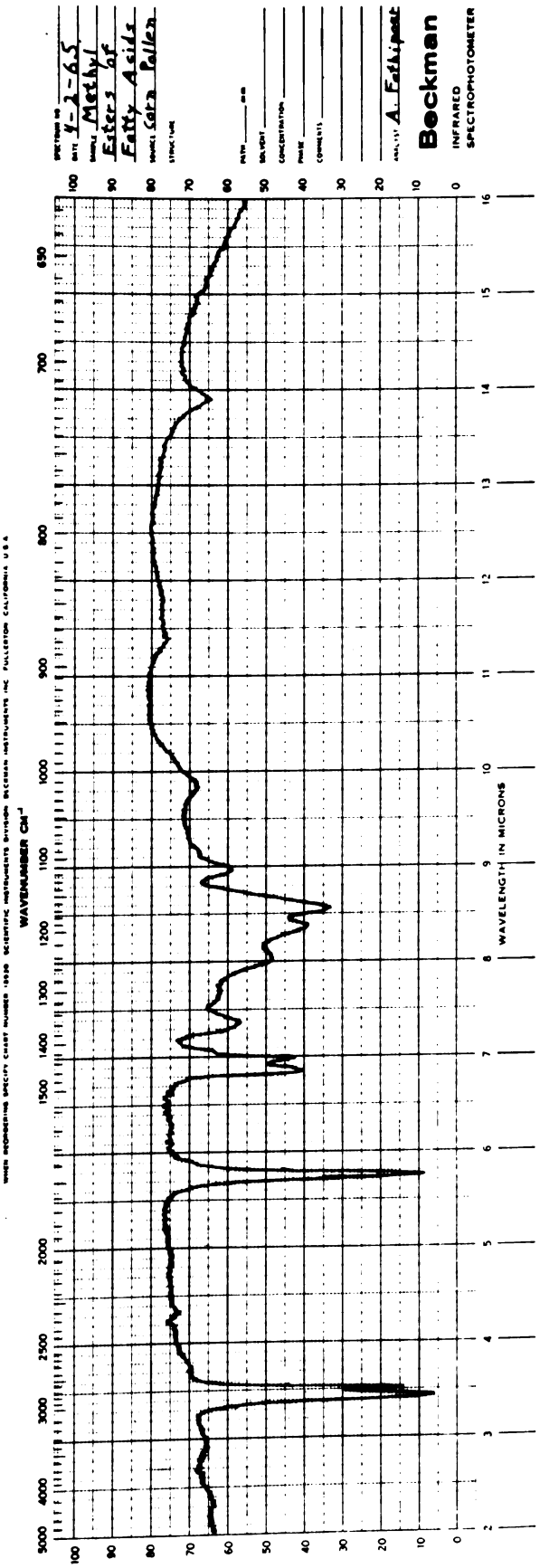
A fraction of the total acids (0.7%) occurred naturally in the form of methyl ester including mainly methyl palmitate and methyl linolenate.

Further evidence for the presence of the naturally occurring methyl esters of the fatty acids was demonstrated by the preparation of chloroform (alcohol-free) or ether (alcohol-free) extracts of pollen. The methyl esters were

obtained in a pure state by utilizing combined column and thin-layer chromatography methods. The identity of the methyl ester was established by infrared spectroscopy and the result is shown in Figure 7. Characteristic absorption maxima were observed at 3.45, 5.75, 8.0, 8.38, and 8.5 microns.

FIGURE 7

IR SPECTRUM OF NATURALLY OCCURRING
METHYL ESTERS


Beckman
INFRARED
SPECTROPHOTOMETER
WAVENUMBER CM⁻¹
WAVELENGTH IN MICRONS
Methyl
Esters of
Fatty Acids
Soya Fallen
A. Fathipour

SUMMARY

The lipid extract of corn pollen was fractionated by column chromatography into phosphatides and nonphosphatides. Nonphosphatides were further fractionated by thin-layer chromatography. Individual fatty acids in each fraction were identified and their concentration estimated by gas-liquid chromatography. The following classes of lipids were found to be present in corn pollen: sterol esters, triglycerides, free fatty acids, free sterols, and other glycerides. One fraction contained methyl esters of longer-chain fatty acids.

The total fatty acids (27 mg.) were obtained after transmethylation of the lipid from one gram of dried corn pollen. Lauric, myristic, myristoleic, arachidic, and behenic acids were identified for the first time. Other acids present were palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic.

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