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GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC IDENTIFICATIONS OF THE HYDROCARBONS AND FATTY ACIDS OF *PLANTAGO OVATA* SEEDS

E. GELPI, H. SCHNEIDER, V. M. DOCTOR,* J. TENNISON† and J. ORÓ

Department of Biophysical Sciences, University of Houston, Houston, Texas 77004, U.S.A.

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Abstract—Gas chromatography and combined gas chromatography and mass spectrometry have been used to study the distribution of fatty acids and hydrocarbons in the seeds of *Plantago ovata*. The occurrence in the seed coat of the low molecular weight hydrocarbons (C_{16}–C_{19}) with a significant percentage of an even carbon-numbered hydrocarbon (anteiso-C_{18}; 10·1 per cent) were unusual features in relation to earlier reports on plant hydrocarbons. The seed coat of *P. ovata* showed mainly linoleic, oleic, and palmitic acid in decreasing concentrations while the seed itself did not contain an appreciable amount of fatty acids. The results rule out a simple precursor-product relationship between fatty acids and hydrocarbons as the likely pathway to explain the biogenesis of all the hydrocarbons.

INTRODUCTION

THIS laboratory has been interested in studying the distribution and biogenesis of hydrocarbons.[1–6] Classical procedures of organic chemistry have proved to be quite inadequate for the separation and analysis of these compounds and it is only during the last 10 years that methods of sufficient sensitivity such as gas chromatography and mass spectrometry have been used for this purpose. Therefore, our knowledge of their distribution in plants is limited. Although it is commonly accepted that one direct route to the plant hydrocarbons involves the decarboxylation of the corresponding fatty acids with one carbon atom more,[7] the results obtained from studies on lipid distributions from this[3,4] and other[8] laboratories do not, in general, find the necessary correlations to support such mechanisms. As an extension of this work, the present report is concerned with the study of the distributions of fatty acids and alkanes in seeds of *Plantago ovata* (Plantaginaceae) using the technique of gas–liquid chromatography and combined gas chromatography–mass spectrometry.[10]

A number of important correlations were reported by Hilditch and Lovern[9] while comparing lipid composition from various species against the phylogenetic scale based on morphological consideration. Based on the occurrence of fatty acids in the seed fats, higher plant

* Present address: Chemistry Department, A & M College, Prairie View, Texas 77445.
† Present address: Shell Chemical Company, Deer Park, Texas 77536.

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families have been classified into four groups.[11] According to this classification, the seeds of the Plantaginaceae belong to group 1b, which includes seeds with linoleic-rich seed fats.[11]

RESULTS

The distribution pattern of hydrocarbons in the seed coat of *Plantago ovata* is shown in Fig. 1 and the percentages are reported in Table 1. The various peaks were identified by gas chromatography and mass spectrometry. In addition to the hydrocarbons in the range

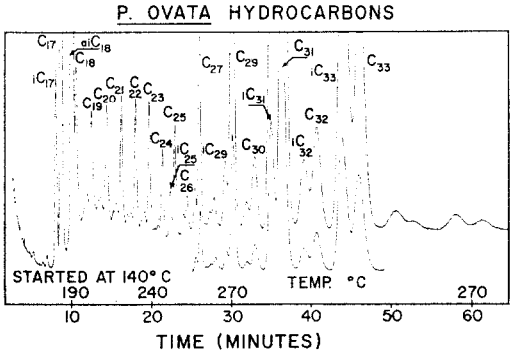

FIG. 1. GAS CHROMATOGRAPHIC SEPARATION OF THE HYDROCARBONS OF *Plantago ovata* SEED COAT
(For details, see Experimental.)

TABLE 1. RELATIVE PERCENTAGE COMPOSITION HYDROCARBONS IN THE SEED COAT OF *Plantago ovata*

Hydrocarbon*	Content (%)	Hydrocarbon*	Content (%)
i-C_{16}	tr.	i-C_{25}	0·4
ai-C_{16}	0·5	n-C_{25}	1·0
n-C_{16}	0·5	n-C_{26}	0·1
i-C_{16}	1·9	n-C_{27}	1·3
n-C_{17}	5·1	i-C_{29}	0·7
i-C_{18}	tr.	n-C_{29}	4·9
ai-C_{18}	10·1	n-C_{30}	1·2
n-C_{18}	1·6	i-C_{31}	8·2
n-C_{19}	1·1	n-C_{31}	20·0
n-C_{20}	2·2	i-C_{32}	1·6
n-C_{21}	2·2	n-C_{32}	2·7
n-C_{22}	1·5	i-C_{33}	10·9
n-C_{23}	1·2	n-C_{33}	9·1
n-C_{24}	0·7		

* The hydrocarbons were identified by their gas chromatographic retention times and by their respective mass spectrometric fragmentation patterns. Symbols: n=normal; i=iso; ai=anteiso. The per cent composition of the hydrocarbons was calculated on the basis of their gas chromatographic area, which was obtained by multiplying the peak heights by the widths at half peak heights. The total hydrocarbon content was 400 ppm. Peaks not identified by mass spectrometry are not included in the Table.

[11] F. B. SHORLAND, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 253, Academic Press, London and New York (1963).

C_{25}–C_{35} predominant in most plant sources,[12] the seeds of *P. ovata* also show the presence of relatively large amounts of lower molecular weight hydrocarbons. Among them, the anteiso-C_{18} is the major component (10·1 per cent). Only two other components, the n-C_{31} and iso-C_{33} occur in higher concentrations (see Table 1).

A close study of the pattern illustrated in Fig. 1. reveals a kind of trimodal distribution for the straight-chain hydrocarbons. The first mode (C_{16}–C_{19}) has its maximum at the n-C_{17}, the second (C_{19}–C_{24}) is centered around the n-C_{21}, while the third (C_{24}–C_{33}) shows a maximum at n-C_{31} as well as a marked predominance of the odd carbon-numbered alkanes. In relation to the methyl substituted hydrocarbons, the first mode is clearly dominated by the anteiso-alkanes, while the iso-alkanes predominate within the third mode.

The seeds themselves contain trace amounts of alkanes as compared with the seed coat, but the pattern is essentially the same. The distribution pattern of fatty acids in the seed coat of *P. ovata* is much less complex than that of the hydrocarbons, as indicated by the identifications and relative percentages reported in Table 2. However, the total hydrocarbon content of this sample (400 ppm) was lower in comparison with the amount of fatty acids (2610 ppm).

TABLE 2. RELATIVE PERCENTAGE COMPOSITION OF FATTY ACIDS IN THE SEED COAT OF *Plantago ovata*

Fatty acid*	Content (%)
Myristic	2·2
Palmitic	10·9
Stearic	2·5
Oleic	16·0
Linoleic	26·3
Linolenic	3·0

* The fatty acids were identified as methyl esters by mass spectrometry and by comparing their retention time with that of authentic samples. The per cent composition of the fatty acids was calculated on the basis of their gas chromatographic areas which were obtained by multiplying the peak heights by the widths at half peak heights. The total lipid content of the seed coat was 2·5 per cent while the methyl esters of fatty acids was 2610 ppm. Peaks not identified by mass spectrometry are not included in the table.

DISCUSSION

Plantago ovata is an annual caulescent herb native to Asia and the Mediterranean countries. This plant is extensively cultivated in India, and of late in Arizona, U.S.A, for its medical applications. In general, the overall distribution pattern of its seed hydrocarbons (see Fig. 1) agrees with the expected predominance of the odd-numbered *n*-alkanes which is common in higher plants.[12] However, it is remarkable that while other higher plants studied contain only traces of low molecular weight hydrocarbons, the major portion of the *P. ovata* seed coat contains significant amounts of hydrocarbons in the C_{16}–C_{22} range. Similar distributions of

[12] G. EGLINTON and R. J. HAMILTON, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 187, Academic Press, London and New York (1963).

lower molecular weight hydrocarbons have been reported in lower plants and microorganisms.[3,5,6,13,14] However, the presence of a large amount of the anteiso-C_{18} alkane constitutes an unusual finding in the plant kingdom.

An alternating pattern in the concentrations of the iso and the anteiso components has been reported by Mold *et al.*[15,16] in wool and tobacco wax where the iso homologs with the odd number of carbon atoms predominated over the corresponding anteiso alkanes and vice versa. This is also the case here (Table 1). Analogous results were reported by Eglinton *et al.*[17] for the high molecular weight alkanes (C_{25}–C_{35}) in the surface wax of the leaves of certain species of *Aconium*; however, the present study appears to be the first report of significant concentrations (20·8 per cent) of hydrocarbons in the C_{16}–C_{19} weight range, with maximum at the anteiso C_{18} alkane, in higher plants.

It is probable that one route to the biosynthesis of plant hydrocarbons involves decarboxylation of the corresponding long-chain fatty acids or their immediate precursors.[7] Mazliak[18] reported that the non-crystalline fraction of the apple cuticle wax contains fatty acids ranging from C_{16}–C_{30}, and the alkanes range from C_{15}–C_{29}. In the present study the results show that one of the pathways for the hydrocarbon synthesis may be the decarboxylation route because C_{18} fatty acids and C_{17} hydrocarbons are both present in significant concentrations. On the other hand, the presence of anteiso-C_{18} alkane (10·1 per cent) cannot be explained on this assumption, because the *P. ovata* seed coat does not contain branched C_{19} fatty acids. Presumably other mechanisms, viz. condensation or the elongation route, which have been discussed by Eglinton and Hamilton,[19] may be involved. This aspect of the study deserves further investigation using tracers.

EXPERIMENTAL

Seed

The husks and the seeds of *Plantago ovata* were obtained from a plantation in Phoenix, Arizona, through the courtesy of Mr. M. D. Rosenthal (P.O. Box 6157, Phoenix, Arizona 85005).

Extraction and Fractionation Procedures

Samples were extracted in a Soxhlet with 50 ml of benzene–methanol mixture (3:1) for 8 hr. The extracts were transferred to beakers, and the solvent was removed by evaporation at 40° in N_2.[1–5] The residue was fractionated on a glass column (1 × 30 cm) with a sintered-glass filter disc filled to a depth of 18 cm with silica gel that had been heat-activated for 24 hr at 410° and then rewashed with *n*-heptane. The first fraction containing the aliphatic hydrocarbons was eluted from the column with *n*-heptane. The second fraction was eluted with benzene, and the third with methanol.

Preparation of Derivatives

The fatty acids were liberated from the glycerides of the methanol fraction by alkaline hydrolysis,[20] and its methyl esters were prepared for GLC analysis as previously described.[21]

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Gas Chromatography

The fractions containing the hydrocarbons and the methyl esters of fatty acids were evaporated in N_2 to a small known volume. The hydrocarbon fraction was separated on a Barber Coleman 5000 gas-chromatograph equipped with a flame ionization detector using a Cu column (3 m × 0·6 cm, i.d.) packed with 1% OV-1 (methyl-silicone gum) on Gas Chrom Q at a N_2 pressure of 700 g/cm², range × 1 and attenuation 10. The methyl esters were analyzed on the same instrument using a stainless-steel column (198 m × 0·076 cm) coated with OV-17 (fluid methyl phenyl silicone) at a N_2 pressure of 2343 g/cm². For identification, a standard solution of authentic samples of fatty acid methyl esters or of a mixture of aliphatic hydrocarbons with pristane and phytane markers were run just prior to running the samples under the same experimental conditions.

Gas Chromatography–Mass Spectrometry

The combined gas chromatographic–mass spectrometric analysis was performed by the use of an LKB 9000 gas chromatograph–mass spectrometer.[10] Mass spectra of the major components of a given mixture were taken instantly as each of these emerged from the gas chromatographic column. The components entered the ion source of the mass spectrometer and were ionized by electron impact at 70 eV for fatty acids and 20 eV for aliphatic hydrocarbons. The ionizing current was set at 125 μA, and the accelerating field, at 3·5 kV. The electron multiplier voltage was adjusted between 1·7 and 2·5 kV according to the size of the peak. Each peak was scanned within the range of 10 to 400 mass units in approximately 10 sec. The spectra were recorded by means of an oscillograph recorder.

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