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HYDROCARBONS IN THE BANANA LEAF, *MUSA SAPIENTUM*

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Abstract—Several long-chain *n*-alkane compounds have been identified by the vapor phase chromatography of urea adducted samples which were prepared from the *n*-hexane eluate fractions of the chloroform–methanol extracts of homogenized leaves. In addition, certain types of alkyl-substituted, fused polycyclic aromatic hydrocarbons have been determined by mass spectrometry, fluorometric, u.v., and i.r. spectroscopy in fractions isolated by preparative thin-layer chromatography from saponified banana leaf extracts. The hydrocarbon composition of most banana leaf extracts differed in part from the hydrocarbon compositions of other plants, namely two xerophytic angiosperms, a moss, a marine alga, a mushroom and two dissimilar red bacteria.

INTRODUCTION

SMALL quantities of hydrocarbons occur in a large variety of natural substances. Early investigations, especially those of Chibnall *et al.*[1] using melting point and X-ray techniques have shown the presence of hydrocarbons in cotton fiber wax. Hydrocarbons have also been detected in the cuticle of apple fruits (*Pyrus malus*),[2] in the waxes from leaves of the carnauba palm (*Copernicia cerifera*),[1,3] in the horsetail (*Equisetum* sp.),[4] and in the cytoplasm of the leaf cells of cabbage and brussel sprouts (*Brassica oleracea*), tobacco (*Nicotiana tabacum*),[1] and other plants. Some of the more recent investigators of plant waxes and hydrocarbons[5–11] successfully used a variety of microanalytical methods including mass spectrometry,[6] gas chromatography,[5] and thin-layer chromatography.[12] It has been shown that in addition to odd carbon number *n*-alkanes even number *n*-alkanes may also be present as minor components.[6] In some species, iso-alkanes appear to be present; this was shown by Eglinton *et al.*,[5] who used elution and gas chromatographic techniques. Iso-alkane compounds have been detected in waxes from rose petals (*Rosa* sp.) and from the leaves of tobacco[6] and of certain members of the family Crassulaceae.[5]

Hydrocarbons also occur in animal tissues, such as in the liver of many fishes,[13] and in beef brain.[14] Hydrocarbons are also present in soils,[15] marine muds,[16] and, of course, in

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petroleum. In spite of their wide occurence in plants and animals, the molecular type composition of several of these hydrocarbons, particularly the aromatics, is not known in detail. Part of the reason for this appears to be the difficulties that used to be inherent in trace hydrocarbon analysis.

Recent application of preparative thin-layer chromatography to the isolation of trace amounts of lipids,[17–19] and an increasing availability of gas chromatography, together with mass spectrometric[20,21] and other types of spectroscopic techniques, make the analysis of trace quantities of hydrocarbons in plant tissues feasible. The purpose of the present study was to apply the new techniques to hydrocarbon and lipid analyses in a variety of plants in order to (*a*) evaluate the limits of the analytical procedure and (*b*) examine the distribution of such compounds in plants. It was observed during this study that banana leaves contained complex waxes of somewhat unusual composition, consequently, the hydrocarbons in this plant were examined in some detail.

Chemical components of the leaves and fruit of the various species of the genus *Musa* have been studied by different investigators. Organic acids, such as L-malic acid[22] and citric acid,[23] have been isolated from the fruit, and sterols and unsaponifiable matter have been reported to be present in banana oil.[24] Anthocyanin pigments were also described from this genus.[25] The distribution of carbohydrates, proteins and amino acids, ascorbic acid and fats in different parts of the leaves of *Musa sapientum* have been examined and a seasonal variation in the concentrations of these components has been observed.[26] A detailed examination, however, of the saturated and aromatic hydrocarbons in the banana leaf has apparently not yet been performed.

EXPERIMENTS AND RESULTS

The n-Alkane Hydrocarbons

The *n*-alkane hydrocarbons were separated and identified by elution and vapor phase chromatography. The leaves were homogenized to extract both cuticular and cytoplasmic lipids. Next, they were extracted with chloroform:methanol 2:1 (v/v) at room temperature. The solutions were evaporated to dryness under N_2. The nitrogen was water pumped, high purity grade gas and it was filtered through a Matheson molecular sieve gas purifier recommended for the removal of water and oil impurities. A part of the residue was redissolved in hexane and eluted on neutral grade Woelm alumina (Grade I) columns, prewashed with *n*-hexane, (and containing at least 250:1 adsorbent to sample, w/w ratios[27]), with the following series of eluents: *n*-hexane, carbon tetrachloride, benzene and methanol. Fifteen 10 ml fractions were collected of each of the eluates and the weights of the evaporated fractions were determined (Fig. 1). The hexane eluate was further purified by rechromatography with *n*-hexane on a Davison silica gel column prewashed with *n*-hexane. The colourless waxy

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residue obtained was analyzed with a Beckman DK-2A spectrometer. The residue showed no absorption bands in the u.v. range indicating the absence of unsaturated impurities in the saturated hydrocarbon fraction. 50 and 100 μg aliquots of the eluate fractions were spotted on thin-layer plates, using silica gel G adsorbent, developed with *n*-hexane, visualized by spraying with Rhodamine 6G indicator, and examined under u.v. light. This procedure served to confirm the presence of saturated hydrocarbons in the hexane eluates from the columns, and the absence of noticeable impurities in the saturated hydrocarbon fraction. A slightly lower spot unresolved from the main fraction was observed in this fraction of the banana leaf extract and is thought to be due to saturated compounds other than *n*-alkanes as indicated by the u.v. spectra. Most of the benzene eluate fraction remained at the origin. Aromatic hydrocarbons can be eluted with benzene from alumina columns[27] and it was found by Murphy *et al.*[19] that some high mol. w. aromatic hydrocarbons and other compounds

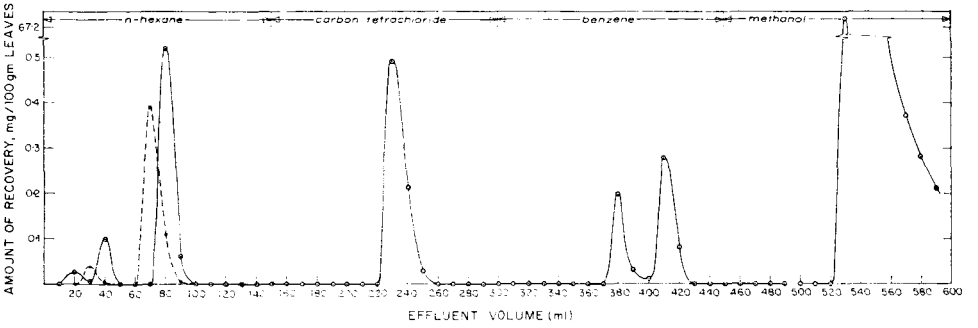

FIG. 1. ELUTION CHROMATOGRAMS OF EXTRACTS FROM TWO BANANA LEAVES (SOLID AND DASHED LINES, RESPECTIVELY).

Woelm, Grade I, alumina column, *n*-hexane, carbon tetrachloride, benzene and methanol eluents. The difference in the positions of the peaks on the chromatograms of the two banana leaves in the *n*-hexane fraction is believed to be caused by different flow rates and differences in packing of the two columns. It has been repeatedly stated in the literature that *n*-hexane elutes most of the saturated hydrocarbons, carbon tetrachloride the unsaturated ones, benzene the aromatic hydrocarbons, and methanol some of the non-hydrocarbon compounds.

remain at the origin when thin-layer plates are developed with hexane. Consequently, the thin-layer chromatograms raised the possibility that aromatic hydrocarbons may also be present in the banana leaves.

Urea adducts were prepared of the hexane eluates by adding a saturated solution of urea in methanol to a benzene–methanol solution of the waxy residue. The resulting adducts were decomposed by heating in water.[28] Urea adduction in general separates straight-chain alkane from branched-chain and cycloalkane fractions. In control experiments using C_{22}, C_{24}, C_{28} *n*-alkane hydrocarbons, most of the samples were recovered after urea adduction. This is in agreement with published data in the literature.[29] The individual *n*-alkane compounds were identified in the saturated hydrocarbon mixtures by vapor phase chromatography using a Barber-Colman, Series 5000, gas chromatograph with a 2 ft long silicone gum rubber (SE-30, 5%) column at 205° isothermal temperature with 120 ml/min flow rate of argon. A tritium, argon ionization detection system was used. The retention times of the hydrocarbons

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extracted from the banana leaf were compared with those of a series of *n*-alkane standards (including internal standards). The presence of C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29} and C_{30} chain length *n*-alkane hydrocarbons was detected in the adducted fraction (iso and cycloalkanes and the shorter chain length *n*-alkanes were not investigated in this study). In the chloroform-methanol extracts of the banana leaves the *n*-alkanes having odd numbers of carbon atoms in their chains were generally far more abundant than the even numbered carbon chain alkanes.

The Aromatic Hydrocarbons

As mentioned above the thin-layer chromatographic data, together with that shown in Fig. 1, raised the possibility that aromatic hydrocarbons may be present in the chloroform–methanol extracts of the banana leaves. Furthermore, in preliminary experiments involving the thin-layer chromatography of saponified banana leaf extracts certain components were detected which might have been hydrocarbons. The possibility was not ruled out that some hydrocarbons were intimately associated with waxes and other saponifiable plant lipids in the leaves and that traces of these hydrocarbons might not be quantitatively separated from the saponified lipid fractions by standard saponification procedures. Consequently, homogenized leaves were extracted in a Soxhlet apparatus with a benzene: methanol mixture 6:4 (v/v) for a period of 6 hr and 10 per cent of KOH in methanol was placed in the flask of the Soxhlet apparatus to saponify lipid components. The potassium salts in the methanol layer in the flask were then extracted with water, acidified with HCl and re-extracted with ether. Infrared spectra were run on the carbon tetrachloride solutions of the extracts in a Perkin Elmer Model 137 spectrophotometer using NaCl optics and 2 mm cavity cells. As expected, the i.r. spectra were characteristic of carboxylic acids, showing strong acid carbonyl bands at 5·8 μ (and what appeared to be ester carbonyl bands at 5·7 μ) as well as shoulders at approximately 2·9 and 3·7 μ and a broad band at 10·7 μ. Silica gel G thin-layer chromatograms of the saponified extracts developed with chloroform: methanol: water 65:25:4 (v/v) showed non-polar components in addition to the usual acids and polar lipids. Thin-layer chromatograms developed with hexane: ether 9:1 (v/v) showed that traces of hydrocarbons were indeed present in the saponified fraction. On thin-layer chromatograms of the saponified extracts of several leaves examined separately a naturally fluorescent spot was visible, which had an R_f value of hydrocarbons in this latter solvent system. This fraction fluoresced bluish-white upon excitation with u.v. light before application of the fluorescent lipid visualizer, Rhodamine 6G. The fluorescent fraction was scraped off the plates, eluted from the silica gel with hexane and analyzed by i.r., u.v., fluorescence, and high mol. wt. mass spectrometry. The i.r. spectra of the fluorescent fraction showed basically a hydrocarbon composition, showing only a trace of a carbonyl band. The fluorescent spectra run in a Beckman DK-2A spectrometer, showed an emission band at 470 mμ and a shoulder at 445 mμ at an excitation radiation of 365 mμ wavelength. Several aromatic hydrocarbons give off bluish colored fluorescence under u.v. light. The u.v. absorption bands, measured in hexane solutions, were detected at 254, 280, 300 and 325 mμ. The u.v. spectral bands are probably caused by a variety of aromatic compounds. For example, 2,3-dimethylphenanthrene absorbs at 255, 280, 285, 298 and 325 mμ, 1-ethylphenanthrene at 256, 277, 288, 300, 318 and 326 mμ and isochrysene at 249, 257, 283, 300 and 320 mμ wavelengths, etc. Saturated hydrocarbons do not absorb in the u.v. range.

The mass spectrometric analysis of high mol. wt. organic compounds is based on the fact that an organic molecule is transformed into a characteristic series of organic ions because of

fragmentation and the loss of a single electron in the mass spectrometer upon bombardment with the electron beam. The instrument sorts out the ions according to their mass to charge (m/e) ratios. The mass spectra of various aromatic and saturated hydrocarbons have been described in the literature[20] as early as 1951. The aromatic rings usually do not show appreciable fragmentation upon electron bombardment. Alkyl branches split off but usually a methylene group remains attached to the aromatic nucleus in one characteristic fragment ion. In the mass spectra of the fluorescent chromatographic fraction a number of organic ions of high ion intensity appeared. For example, high ion intensities at m/e (where $e = 1$) ratios of 128 and 141 were measured; these can be caused by the naphthalene nucleus (mol. wt. = 128) and naphthalene with one methylene branch attached (mol. wt. = 141), respectively. High ion intensities at the m/e ratios of 178 and 191 may be the fragmentation products of an alkyl substituted, fused tricyclic aromatic compound. These ions may be, for example, phenanthrene (mol. wt. = 178) and phenanthrene with one methylene branch attached (mol. wt. = 191), respectively. The mass spectra of the hydrocarbon fraction in general indicated an approximately equal mixture of saturated and of alkyl aromatic compounds. Naphthalenes, phenanthrenes and/or anthracenes, pyrenes and possibly benzoanthracenes or chrysenes seemed to be the predominant components. The highest mol. wt. component had a mass to charge (m/e) ratio of 467. In an attempt to partially verify the mass spectra the fluorescent substance was further fractionated by silica gel G thin-layer chromatography, at this time using the non-polar *n*-hexane as the developing solvent. This resulted in the separation of at least seven types of hydrocarbon groups, the naturally fluorescing spot having now an R_f value similar to hydrocarbons containing 6–8 double bonds. Phenanthrene has 7 conjugated double bonds. Thus, the chromatogram was in agreement with the mass and u.v. spectra.

DISCUSSION

Plant waxes occur in the leaf cuticle, where they form wax platelets in a mixture of cutin and pectin[8] and as wax rodlets extruded to the surface. Waxes are also present in fruit cuticles and seed coats and they may be dispersed in the cell in the same way as fats. No attempts were made in the present study to detect the locations where hydrocarbons occur in the leaves or to examine their seasonal variation.

The role and physiological significance of hydrocarbons in banana leaves is not fully understood. The long-chain *n*-alkanes are undoubtedly indigenous constituents of the leaves. The aromatic hydrocarbons, including the bluish-white fluorescent component which is apparently an alkyl-aromatic hydrocarbon, are probably indigenous. Even though the saponification conditions were mild, there is a possibility that some of the aromatic hydrocarbons may be artifacts, synthesized from other compounds by some reaction during the chemical isolation process. Should this be their origin, a knowledge of the aromatic hydrocarbon types may help to elucidate the structures of their parent compounds in the waxes.

The possibility that the aromatic hydrocarbons are the result of contamination from polluted air should also be considered. The banana leaves were obtained from plants near the shore of the Pacific Ocean in La Jolla, California, which is a non-industrial community. Some of the other plants examined in this study, such as the fungus *Pholiota praecox*, were also collected in La Jolla and they did not contain the fluorescent, aromatic hydrocarbon component. It appears that at least this aromatic substance is an indigenous component of the banana leaf, although its concentration may be subject to seasonal variations. Furthermore, the naturally fluorescent aromatic hydrocarbon occurred only in the saponified fractions and not in cold chloroform–methanol extracts of the banana leaves. This would further suggest

that this component is not an air pollution product on the leaf surfaces because if it were such it should have been possible to remove it by cold chloroform–methanol washing.

The naturally fluorescent polycyclic aromatic hydrocarbon component was not detected by thin-layer chromatography in the saponified extracts of other plants (with the possible exception of the moss *Polytrichum commune*, which showed traces of what seemed to be a similar component). The bacteria *Chromatium* sp. and *Saprospira grandis*, the brown alga *Pelagophycus porra*, the fungus *Pholiota praecox*, the cactus *Opuntia echinocarpa*, and the ocotillo, *Fouquieria splendens*, all showed traces of hydrocarbons on their thin-layer chromatograms but lacked the fluorescent component. The present study provides further evidence that hydrocarbons occur in plants and it further demonstrates the applicability of recently developed spectroscopic and chromatographic procedures to plant hydrocarbon analysis.

CONCLUSIONS

The combined techniques of elution, thin-layer and gas chromatography, i.r., u.v., fluorometric and mass spectrometry revealed several hydrocarbon components in banana leaves, *Musa sapientum*. Several *n*-alkane and some aromatic hydrocarbons were detected. Naturally fluorescent compound(s), which appear(s) to be a type of alkyl substituted polycyclic aromatic hydrocarbon, is a noteworthy component of the saponified extracts. This work may support earlier findings and subsequent suggestions, such as those made by Eglinton *et al.*[5] and Purdy and Truter,[12] regarding the possibility of using variations in plant hydrocarbon and lipid compositions for taxonomic purposes.

EXPERIMENTAL

Experimental Controls

The experiments were performed in glassware cleaned with a 85:15 (v/v) mixture of hot concentrated sulfuric and nitric acids prior to use. No rubber or plastic implements or stopcock grease were employed in the analytical procedure. Blank runs were performed prior and parallel to all experiments to detect laboratory contaminations if such should have occurred. All solvents were spectral grade and, in addition, they were freshly distilled through 12 in. columns packed with Raschig rings prior to use. Solvents contained less than 0·4 mg/100 ml residue, and showed no extraneous i.r. absorption bands. These precautions helped to ensure that the hydrocarbons were indigenous components of the banana leaves and not laboratory contaminations.

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