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REVIEW ARTICLE
ALIPHATIC HYDROCARBONS OF THE FUNGI*

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Abstract—The presence of paraffinic hydrocarbons throughout the plant and animal kingdom has received considerable attention during the past decade. Hydrocarbons have been recently reported for the spores of phytopathogenic fungi and are found to be structurally very similar to the alkanes of higher plants. It appears that the hydrocarbon components of the few mycelial and yeast forms reported resemble the distribution found in bacteria. The occurrence and distribution of these compounds in the fungi are reviewed.

ALIPHATIC hydrocarbons have been detected in most every plant, animal and microorganism examined. The distribution and metabolism of higher plant[1–4] and bacterial[5] hydrocarbons have been the subject of several reviews during the past 3–4 yr. Paraffinic hydrocarbons are associated with the epicuticular waxy coating of higher plant surfaces and were first identified by crude chemical methods as early as 1929.[6–8] The early investigations have been confirmed and expanded using more sophisticated instrumentation such as gas–liquid chromatography and GLC–MS. The complex nature of this waxy coating was revealed with the identification of lipid components such as long chain fatty acids, primary and secondary fatty alcohols, waxy esters, ketones, aldehydes, acetals, diols, terpenes, glycerides and others.

Normal, *iso*- and ante*iso*-branched, and unsaturated paraffinic hydrocarbons are found widely distributed throughout the plant kingdom often representing up to 15% of the total dry mass of certain plant tissues. Typical hydrocarbon distributions range from C_{16} to C_{35} with the odd-numbered carbon chain-lengths predominant. Generally, the principal carbon chain-lengths are C_{29}, C_{31} and C_{33}. Even-numbered and branched carbon chains and alkenes are often found in lesser concentrations. Chain-length predominance, branching, and the presence of unsaturation varies considerably depending on the environment, plant tissue and stage of development.

Alkanes are also present in various blue-green and green algal species. Distributions similar to higher plants are found in several species, while others also have predominant hydrocarbons of shorter chain-lengths. For example, C_{17} normal and branched (7- and 8-methyl) alkanes are common in *Anabaena variabilis*[9] and other species.[10,11]

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[1] G. EGLINTON and R. J. HAMILTON, *Science* **156,** 1322 (1967).
[2] P. E. KOLATTUKUDY, *Lipids* **5,** 259 (1970).
[3] P. E. KOLATTUKUDY, *Science* **159,** 498 (1968).
[4] P. E. KOLATTUKUDY, *Ann. Rev. Plant. Physiol.* **21,** 163 (1970).
[5] P. W. ALBRO and J. C. DITTMER, *Lipids* **5,** 320 (1970).
[6] A. C. CHIBNALL, S. H. PIPER, A. POLLARD, R. E. WILLIAMS and P. N. SAHSI, *Biochem. J.* **28,** 2189 (1934).
[7] E. CRENSHAW and I. SMEDLEY-MACLEAN, *Biochem. J.* **23,** 107 (1929).
[8] A. C. CHIBNALL and S. H. PIPER, *Biochem. J.* **28,** 2209 (1934).
[9] W. G. FEHLER and R. J. LIGHT, *Biochem.* **9,** 418 (1970).
[10] H. SCHNIEDER, Dissertation University of Houston, Houston, Texas (1969).
[11] K. WINTERS, P. L. PARKER and C. VANBAALEN, *Science* **163,** 467 (1969).

The hydrocarbon components of a single plant are not restricted to the extra-cuticular surface. For example, chloroplast preparations of *Antirrhinum majus* leaves contain alkanes that differ in relative concentrations from leaf surface alkanes of the same plant.[12] After first removing the surface waxes, Kaneda[13] found hydrocarbons in leaf tissues which differed from the external paraffiins in carbon chain-length range, predominance, and concentration. Weete *et al.*[14] reported hydrocarbons within the cells of non-differentiating tobacco tissue cultures that were different from the tobacco leaf cuticular alkanes. Principal internal alkane carbon chain lengths were C_{22}, C_{23} and C_{24}. Characteristically, all the internal alkanes so far found are present in very low concentrations, their carbon chain-lengths range from C_{16} to C_{28}, and there is no odd-numbered carbon chain-length predominance. Internal alkanes have also been found in other higher plant species.[15]

Aliphatic hydrocarbons are also found in animal[3,16] and bacterial[5,17–19] systems. Their distribution in animals is very similar to that of higher plants while in bacteria a very complex mixture of normal, singly and doubly branched, and unsaturated structural isomers is present. The various branched-chain isomers include *iso*, ante*iso*, *iso*–*iso*, *iso*–ante*iso* and ante*iso*–ante*iso* configurations. Of the few bacterial species examined, interesting variations in the hydrocarbon distributions for each are shown. For example, *Vibrio marinus* paraffin chain-lengths range from C_{15} to C_{18} while in two strains of *Sarcina lutea* the carbon chains range from C_{23} to C_{30}.

In 1965, Baker and Strobel[20] first reported the presence of aliphatic hydrocarbons in the fungi by identifying a homologous series of *n*-alkanes extracted from the uredospores of *Puccinia striiformis*. Quite independently the following year, Oró *et al.* reported similar alkane components in the chlamydospores of several smut fungi.[21] These reports stimulated investigations to determine the distribution of alkanes in the dormant spores of selected phytopathogenic fungi.[22–26] Generally, fungal spore alkane carbon chain lengths range from C_{18} to C_{35} while the principle hydrocarbons are C_{27}, C_{29} and C_{31} (Table 1). Alkanes having odd-numbered carbon chains are predominant while even-numbered carbon chains are present in lesser concentrations. Branched-chain isomers are reported for some species.

Eglinton and Hamilton[1] related that hydrocarbon distributions of higher plants were sufficiently complex that they might provide a fingerprint by which plant taxa could be delimited. Oró *et al.*[21] suggested that alkane distributions of fungal spores may serve as a taxonomic character to distinguish plant pathogens. Thus, the spore hydrocarbon distributions of several fungal species were analyzed to determine their potential as a chemotaxonomic tool. Qualitatively, the alkane components of the rust and smut species studied

[12] P. GÜLZ, *Phytochem.* **7,** 1009 (1968).
[13] T. KANEDA, *Phytochem.* **8,** 2039 (1969).
[14] J. D. WEETE, S. VENKETESWARAN and J. L. LASETER, *Phytochem.* **10,** 939 (1971).
[15] G. A. HERBIN and P. A. ROBINS, *Phytochem.* **8,** 1985 (1969).
[16] R. F. N. HUTCHINS and M. M. MARTIN, *Lipids* **3,** 250 (1967).
[17] T. G. TORNABENE and S. P. MARKEY, *Lipids* **6,** 190 (1971).
[18] P W. ALBRO, T. D. MEEHAN and J. C. DITTMER, *Biochem.* **9,** 1893 (1970).
[19] T. G. TORNABENE, E. GELPI and J. ORÓ, *J. Bacteriol.* **94,** 333 (1967).
[20] K. BAKER and G. A. STROBEL, *Proc. Mont. Acad. Sci.* **25,** 83 (1965).
[21] J. ORÓ, J. L. LASETER and D. J. WEBER, *Science* **154,** 399 (1966).
[22] J. D. WEETE, J. L. LASETER, D. J. WEBER, W. M. HESS and D. L. STOCK, *Phytopathol.* **59,** 545 (1969).
[23] J. L. LASETER, W. M. HESS, J. D. WEETE, D. L. STOCK and D. J. WEBER, *Can. J. Microbiol.* **14,** 1149 (1968).
[24] J. D. WEETE, D. J. WEBER and J. L. LASETER, *J. Bacteriol.* **103,** 536 (1970).
[25] J. D. WEETE, D. J. LETOURNEAU and D. J. WEBER, *Arch. Für Microbiologie* **75,** 59 (1971).
[26] J. D. WEETE, Dissertation, University of Houston, Houston, Texas (1970).

TABLE 1. RELATIVE HYDROCARBON DISTRIBUTION IN THE THE SPORES OF CERTAIN PHYTOPATHOGENIC FUNGI[21,22,25]

Species	Hydrocarbon chain-length																	
	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	iC_{25}	C_{26}	C_{27}	iC_{27}	C_{28}	C_{29}	iC_{29}	C_{30}	C_{31}	iC_{31}	C_{32}	C_{33}
Tilletia foetida	—	—	0·3	0·3	3·2	1 0	11 9	—	1·3	24·7	0·9	5·7	28 8	1·6	1·9	15·2	1·3	—	1 7
T. caries	—	—	0·1	0·2	5 4	0·9	9·7	—	1 0	23·5	1·0	4·3	34·8	1·3	1·6	13·7	1·1	—	1 5
T. controversa	—	—	0·4	0 2	3·9	0·9	11·4	—	0·4	24 7	0·8	2·7	25 6	1·9	1·3	21·5	1·7	—	2 7
Puccinia graminis var. tritici	—	—	—	—	3 1	7·2	7·3	—	8·1	17 6	—	7·3	29 4	—	1·3	16·2	—	—	2 0
P. striiformis	—	—	—	—	3·9	3·2	7·9	—	2·2	20·5	—	2·5	23 9	—	—	23·9	—	—	3·4
Sphacelothica reiliana	—	—	—	—	2·1	—	3·2	1·8	1·1	9·1	2·8	3·8	34 2	8·3	13·1	16·2	5 1	2·7	2 5
Ustilago maydis	1·1	1·5	—	—	1·9	1·3	11·1	—	2·1	33·4	8·4	7 7	13·8	6·9	3 8	—	3 1	1 7	3·6
Urocystis agropyii	1·1	—	1·5	—	2·0	1·2	3·3	1·6	1·0	12·2	—	0·9	46·3	—	1·2	23·7	—	—	—

were very similar. For example, three closely related *Tilletia* species (*foetida, caries* and *controversa*) could not be distinguished by qualitative comparison of the gas chromatographic separation patterns (Fig. 1), while only slight differences were noted between the *Tilletia*, *Puccinia* and *Urocystis* species examined. When analyzed quantitatively, however, distinct differences were noted in both major and minor alkane components (Table 1).

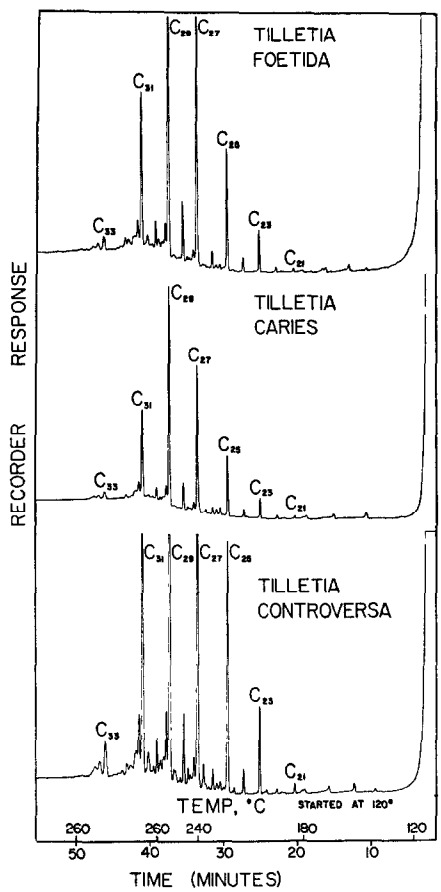

FIG. 1. COMPARISON OF THE PARAFFINIC HYDROCARBON DISTRIBUTION OF THE *Tilletia* SPECIES OBTAINED FROM INFECTED WHEAT KERNELS.[22]

Laseter and Valle[26a] have recently reported an alkane distribution for uredospores of *P. graminis avenae*, which differs considerably from that of *P. graminis tritici*. While C_{23} was the principal hydrocarbon of *P. graminis avenae*, C_{29} was predominant in *P. graminis tritici*. Also, a single race (6AF stem rust) and a mixture of leaf rust races could be readily distinguished. For example, nC_{23} represented over 50 mole% of the leaf rust hydrocarbons while C_{23}, C_{25}, C_{27} and C_{29} ranged from 14 to 17% each in the stem rust spores. Total hydrocarbon concentrations ranged from 40 to 150 ppm for various rust and smut species.[21, 26a] These differences could be characteristic and used as a chemotaxonomic character to delimit fungal species. Before definite conclusions can be drawn concerning the taxonomic value of fungal spore alkanes, the extent of variation from field to field and from season to season should be established while many more species from defined media and natural host tissues must be examined.

The detection of paraffinic hydrocarbons in fungal spores suggested the presence of a waxy coating analogous to that surrounding exterior surfaces of many higher plant tissues. Electron microscopic studies using the freeze-etch method did not, however, reveal the presence of such a coating exterior to the spore wall (Figs. 2 and 3).[22,23] No differences in the exterior spore wall architecture were observed after extraction with organic solvents[23] or in published scanning electron micrographs.[27]

Thus, the exact origin and location of fungal spore hydrocarbons are not yet certain. Each of the above fungal species for which spore hydrocarbons are reported was a plant pathogen and spores for analysis were obtained from the host tissues. Since caution was taken to prevent contamination by petroleum products and host tissue fragments, it is possible that fungal spore hydrocarbons are taken up during sporulation or they are a product of the host–parasite interaction. For example, Laseter and Weber[28] found the relative alkane distributions different for *Ustilago maydis* chlamydospores, and infected and uninfected corn kernels. On the other hand, Weete *et al.*[23] found the alkane distributions of *T. caries*, *T. foetida*, *T. controversa* and uninfected host wheat kernels qualitatively identical. If hydrocarbons are present in the spores of many fungal species, their intracellular distribution is also uncertain. Recent evidence suggests that differences in relative lipid compositions may exist within the spore. For example, differences were noted for the spore wall and protoplast fatty acids in *T. controversa*[29] and *P. graminis avenae*.[26a]

Jones[30] reported that paraffinic hydrocarbons were present in the mycelia of three fungal species grown in aeriated liquid media. *Penicillium sp.* and *Aspergillus sp.* contained homologous series of alkanes ranging in carbon chain lengths from nC_{15} to nC_{36} with major components ranging from nC_{27} to nC_{30}. *Trichoderma viride*, on the other hand, possessed a similar chain length range but a significantly different relative distribution with the predominant alkanes being nC_{22}, nC_{24}, and an unknown compound in increasing concentrations, respectively. It is interesting to note that no significant odd-carbon chain length predominance is found in the *Penicillium* and *Aspergillus* species while *T. viride* contained an odd/even ratio less than one. Although each of the species were identically cultured, no alkane analyses were presented for the non-defined and partially defined media employed in the above study. Weete *et al.*[24] found no hydrocarbons in mycelia grown in liquid media nor in sporangiospores of *Rhizopus arrhizus* harvested from cultures grown on defined solid

[26a] J. L. LASETER and R. VALLE, *Environ. Sci. Technol.* **5,** 631 (1971).
[27] F. J. SCHWINN, *Phytopathol.* **2,** 376 (1969).
[28] J. L. LASETER and D. J. WEBER, *Phytopathol.* **58,** 886 (1966).
[29] E. J. TRIONE and T. M. CHING, *Phytochem.* **10,** 227 (1971).
[30] J. G. JONES, *J. Gen. Microbiol.* **59,** 145 (1969).

media. The same results were obtained from sclerotial analyses of *Sclerotium sclerotinium* collected from both defined media and natural host tissues.[25,26]

Hydrocarbons have also been reported for several yeast species. Alkanes ranging in carbon chain lengths from C_{16} to C_{39} were reported for *Debaryomyces hansenii*, but the identifications were not confirmed.[31] Merdinger *et al.*[31a] reported hydrocarbon components for *Pullularia pullulans* which were very similar to that of bacteria. They identified a complex pattern of saturated and unsaturated homologues ranging from C_{16} to C_{28}. Several branched-chain isomers were also present. The predominant carbon chain lengths were straight-chained C_{19} to C_{22} hydrocarbons. A very similar distribution was reported for the yeast *Candida utilis* grown in defined media under anaerobic conditions.[32] A homologous series of alkanes ranging in chain length from nC_{14} to nC_{29} was found in the cytoplasmic membrane (10·0 mg), cellular contents (0·4 mg) and external to the cell wall (0·5 mg). Monounsaturated alkenes (C_{16} to C_{23}) were also found in hydrocarbon fractions from each source. Again no odd-numbered carbon chain length predominance was present in this species. It is interesting to note that a polyunsaturated alkene, hexadecatriene, was identified in each fraction while squalene (over 50 per cent of the hydrocarbons) was present in only the plasma membrane fraction. In another yeast species, *Saccharomyces sp.*, alkane chain lengths ranging from C_{15} to C_{34} were reported while no alkenes were identified.[30]

As in higher plants, the function(s) of fungal hydrocarbons is not known. Suggested functional roles of fungal spore alkanes include, (1) the prevention of desiccation and insulation from extreme temperatures, (2) resistance to microbial attack, (3) inhibition of germination, and/or (4) a role in the infection process. The fact that membrane elements of *C. utilius* contains over 20 times the hydrocarbon concentration of other cellular and extracellular components and that squalene is present suggests a possible role in membrane structure and the site of synthesis. These are certainly areas that should be explored.

If, indeed, the paraffinic hydrocarbons are indigenous to the fungal spores, their chain-length distribution and relative abundances are very similar to higher plant cuticular alkanes. However, on the basis of the very few reports to this date, hydrocarbon distributions of fungal mycelia and particularly of yeast forms may be similar to that of bacteria. Before definite conclusions concerning the universal occurrence and nature of aliphatic hydrocarbons in the fungi can be drawn, many more species grown on standardized and defined media must be studied.

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[31a] E. MERDINGER and E. M. LEVINE, JR., *J. Bacteriol.* **89,** 1488 (1965).
[31] E. MERDINGER, P. KOHN and R. C. MCCLAIN, *Can. J. Microbiol.* **14,** 1021 (1968).
[32] M. FABRE-JONEAU, J. BARAUD and C. CASSAGNE, *Compt. Rend.* 268 (1969).

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