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TWO POPULATIONS OF ALIPHATIC HYDROCARBONS OF TERATOMA AND HABITUATED TISSUE CULTURES OF TOBACCO

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Abstract—Teratoma and habituated tissue cultures of tobacco grown under identical conditions were examined for the presence of paraffinic hydrocarbons. The teratoma tissues contained *n*-C_{29}, 2-methyl C_{30} (*iso* C_{31}) and *n*-C_{31} as the major alkane components and their distribution pattern was qualitatively identical to the seedling tissue alkanes (C_{22}–C_{34}). Habituated tissues contained a different population of alkanes ranging in carbon chain length from C_{17} to C_{28}. The predominant alkane components were *n*-C_{23}, *n*-C_{22}, and *n*-C_{24} in decreasing concentrations respectively. A tissue culture system is presented where the Population I hydrocarbons (C_{16}–C_{28}) are synthesized separately and independently of Population II hydrocarbons (C_{27}–C_{34}).

INTRODUCTION

PARAFFINIC hydrocarbons are found in tissues of plants from all divisions of the plant kingdom. The distribution and metabolism of these compounds in plant tissues have been extensively reviewed,[1–5] but the exact physiological importance of these hydrophobic components remains uncertain.[5] Emphasis has been placed on the hydrocarbon components as part of waxy layers on the external surfaces of leaves, stems, and floral parts exposed to the atmosphere. Only recently has attention been given to the internal population of relatively short chain alkanes.[6]

The surface and cellular alkanes of differentiated plant systems have received greatest attention whereas those of non-differentiated tissue cultures have not been examined. The teratoma and habituated tissue cultures of tobacco represent excellent systems to illustrate the metabolic differences between differentiated and non-differentiated tissues. The teratoma tissue is of tumorous origin (crown gall disease caused by *Agrobacterium tumefaciens*) and maintains its pathological nature under normal conditions of culture while having a large degree of differentiation (shoots and leaves). The habituated tissue is of normal origin, only proliferates, and does not show any organ differentiation.[7,8]

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This paper reports the presence and distributions of two populations of paraffinic hydrocarbons as they occur separately in the teratoma and habituated tissue cultures of tobacco. The developmental aspects of tissue cultures of tobacco with respect to hydrocarbon synthesis are discussed.

RESULTS AND DISCUSSION

The paraffinic hydrocarbons of 4–6-week-old tobacco seedling tissues are both qualitatively and quantitatively similar to that reported previously by Kaneda.[9,10] There are three homologous series consisting of the normal, 2-methyl, and 3-methyl substituted branched-chain alkanes. A unique pattern of branched-chain isomers exists. Methyl substitution at the number two position is found only in the odd-numbered carbon compounds while methyl substitution at the number three position occurs only in the even-numbered carbon compounds.

Total hydrocarbon concentrations of the tobacco seedling and teratoma tissues are 1557·0 and 156·8 μg/g dry wt. respectively. The ratio of normal to branched-chain alkanes in the seedling and teratoma tissues is approximately 1·1:1 and 2:1 respectively, revealing that teratoma tissues contain higher relative concentrations of straight chain isomers than the seedling tissue.

The three predominant hydrocarbon components of seedling tissues used for this study are n-C_{31}, n-C_{33}, and 3-methyl C_{31} (2-methyl C_{30} is found in concentrations slightly less than 3-methyl C_{31}) in decreasing concentrations respectively (Table 1). This corresponds closely to the results obtained by Kaneda[9,10] who found that n-C_{31}, 2-methyl C_{30}, and n-C_{33} in decreasing concentrations respectively were the predominant alkanes. The slight variation in relative proportions is probably due to differences in age of the tissues and methods of extraction.

The hydrocarbon fraction of teratoma tissue culture samples contained a distribution of alkanes identical to the seedling tissues. The predominant components of this tissue were n-C_{29}, 2-methyl C_{30}, and C_{31} in decreasing concentrations respectively (Table 1). This is similar to the seedling with the exception that n-C_{29} appears in the highest concentrations while n-C_{33} is not a major component of the teratoma tissues. With the exception of 3-methyl C_{31}, odd-numbered carbon compounds are predominant in both the seedling and teratoma tissues. The predominance of odd-numbered hydrocarbon chain lengths is typical of most higher plant species.[2–5]

Past studies of the hydrocarbon distribution patterns have concentrated on the epicuticular waxes of higher plants. Recently Kaneda[6] has reported the internal hydrocarbons of spinach leaf tissues. These alkanes were obtained from the total lipid extract of macerated leaf tissues after removal of the external waxes by the dipping method. The alkanes obtained by this method were found in much lower relative proportions than the alkanes of the cuticular waxes ranging in carbon chain length from C_{16} to C_{28}. Characteristics of the distribution pattern of these internal alkanes differs somewhat from those of the surface alkanes. The predominant compounds of the internal hydrocarbons are in the C_{22}–C_{24} carbon chain length range. The predominant high odd over even-numbered carbon chain length ratio common in surface waxes is not present.

When the hydrocarbon components from total lipid extracts of habituated tissue culture samples were examined, a distribution was found which is very similar to that reported for

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TABLE 1. HYDROCARBON COMPONENTS OF HABITUATED AND TERATOMA TISSUE CULTURES AND SEEDLING TISSUES OF TOBACCO

Hydrocarbon	Habituated		Teratoma		Seedling	
	%	μg/g*	%	μg/g*	%	μg/g*
C_{17}	4·8	2·0	—	—	—	—
C_{18}	5·7	2·5	—	—	—	—
C_{19}	4·6	2·0	—	—	—	—
C_{20}	7·6	3·5	—	—	—	—
C_{21}	6·9	3·0	—	—	1·0	16·1
C_{22}	12·3	6·0	1·6	2·4	0·8	10·7
C_{23}	19·1	9·5	3·5	5·5	1·0	16·1
C_{24}	11·8	5·5	3·2	5·0	0·9	14·3
C_{25}	8·1	3·5	3·3	5·3	1·2	19·8
C_{26}	5·4	2·5	3·2	5·0	0·9	14·3
2-Me-C_{26}	—	—	1·5	2·4	trace	trace
C_{27}	5·0	2·0	5·9	9·5	4·8	75·0
3-Me-C_{27}	—	—	2·0	3·2	1·8	28·6
C_{28}	5·2	2·5	6·0	9·5	1·9	30·3
2-Me-C_{28}	—	—	9·5	15·2	7·5	118·0
C_{29}	—	—	12·3	18·2	8·6	135·5
3-Me-C_{29}	—	—	7·2	11·4	9·4	147·5
C_{30}	—	—	4·3	6·6	3·0	47·4
2-Me-C_{30}	—	—	10·9	17·2	10·1	158·7
C_{31}	—	—	10·3	16·4	14·7	228·2
3-Me-C_{31}	—	—	5·0	7·9	10·4	164·0
C_{32}	—	—	2·8	4·5	3·1	47·7
2-Me-C_{32}	—	—	3·2	5·0	5·1	79·8
C_{33}	—	—	4·2	6·6	12·5	195·0
3-Me-C_{33}	—	—	trace	trace	trace	trace
C_{34}	—	—	—	—	trace	trace
Total hydrocarbon		44·5		156·8		1557·0

* μg alkane/g dry wt. of tissue.

the internal alkanes of spinach leaf tissues. Very low concentrations of alkanes (44·5 μg/g dry wt. of tissue) were present (Table 1). Alkanes were present in concentrations less than 1/3 that of the teratoma tissue alkanes and 1/35th that of the seedling tissue alkanes. Carbon chain lengths range from C_{17} to C_{28} with no predominance of the odd-numbered carbon chain lengths (Table 1). Major alkane components of habituated tissue culture samples are C_{23}, C_{22} and C_{24} in decreasing concentrations respectively (Table 1). Several compounds were resolved by GLC in such low concentrations that structural confirmations could not be made. These components were located between the major alkanes of the chromatogram and probably represent specifically branched or unsaturated isomers.

Comparison of the hydrocarbon fractions of the partially differentiated teratoma tissues and seedling tissues reveal no qualitative differences while teratoma tissues contain significantly less alkanes than the seedling tissues. On the other hand, alkanes of the non-differentiated habituated tissues differ both qualitatively and quantitatively, from the teratoma and seedling tissues. A distribution of hydrocarbons almost identical to the internal hydrocarbons of spinach leaves reported by Kaneda[6] was found. Internal hydrocarbon distributions are probably of universal occurrence but have been overlooked because of the

methods of extraction and that high concentrations of higher molecular weight alkanes mask their presence during analysis. For example, Weete *et al.*[11] found a very similar distribution of alkanes in the root tissues of the halophyte *Salicornia bigelovii* which has a cortical surface with no cuticular waxy coating. Thus it appears that many higher plants contain two populations of hydrocarbons: Population I which are found in low concentrations with carbon chain lengths of C_{16}–C_{28} with no odd-numbered carbon chain predominance and located internal to the leaf surface and Population II, located in the external waxy coating and consisting predominantly of higher molecular weight alkanes with their major components containing odd-numbered carbon chain lengths.

A comparison of the hydrocarbon distributions of the teratoma (differentiated) and habituated (non-differentiated) tissues illustrates this two population concept and further suggests the presence of two enzyme systems for their synthesis and/or two loci of synthesis. A composite of the teratoma and habituated tissue hydrocarbon components reveals a bimodal distribution: Population I of the habituated and Population II of the teratoma tissues. The non-differentiated habituated tissues apparently does not contain the enzyme system or metabolic machinery for activation of an enzyme system necessary for synthesis of the Population II hydrocarbons.

In summary, the data presented in this paper demonstrates the presence of two individual populations of paraffinic hydrocarbons appearing separately in teratoma and habituated tissue cultures of tobacco. Of greater significance, however, is that a tissue culture system is presented that is capable of synthesizing only the Population I hydrocarbons providing an ideal system for answering questions concerning the developmental aspects of activation and localization of the biogenesis of paraffinic hydrocarbons.

EXPERIMENTAL

Tissue cultures and seedlings. Teratoma and habituated tissue cultures were grown by the methods previously described by Chen and Venketeswaran.[8,12,13] Seedling tissues (4–6-week-old) were grown in soil with 12 hr alternating illumination. All tissues were harvested, dried by lypholyzation, weighed, and stored at − 4° prior to lipid extraction.

Extraction and sample preparation procedures. 1g dry wt. of the lypholyzed teratoma and habituated tissues and 0·42 g (dry wt.) of the seedling tissues were extracted for total lipids by the methods described by Weete *et al.*[14] The total lipid extract was subjected to alkaline hydrolysis described by Wilde and Stewart.[15] The hydrolysate was first washed with 3 vol. (20 ml each) of *n*-heptane to remove the non-saponifiable material. The lower aqueous phase was adjusted to pH 2 and washed with 3 vol. (20 ml each) Et_2O. Both organic phases were taken to dryness under N_2 with gentle heating. The non-saponifiable fraction was taken up in *n*-heptane and placed on top of a pretreated (150° for 1 hr) silica gel column (1 × 20 cm). The hydrocarbon components were eluted with 3 vol. (20 ml each) of *n*-heptane. The hydrocarbon fraction was taken to dryness prior to analysis by gas chromatography and GLC-mass spectrometry combination.

GLC and GLC-mass spectrometry. The hydrocarbon fraction was taken up in 20–50 μl of *n*-heptane and 1/20–1/50 of the total fraction was injected directly into a Perkin Elmer 900 gas chromatograph equipped with a 15·2 m × 0·076 cm stainless steel capillary column coated with SE-30 (Applied Science Laboratories, State College, Pa., U.S.A.). The oven temperature was programmed from 100° to 250° at 10°/min. Solvent blanks were run to ensure the absence of contaminants.

Structural confirmation of each paraffinic hydrocarbon was made by mass spectrometry. The mass spectra of the alkanes were identical to those previously published.

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