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HYDROCARBON AND FATTY ACID DISTRIBUTION IN THE HALOPHYTE, *SALICORNIA BIGELOVII*

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Abstract—The paraffin hydrocarbons and total fatty acids extracted from *Salicornia bigelovii*, collected from two distinct ecological areas of the Texas Gulf Coast, were analyzed by TLC and GLC. Although qualitatively these lipid components were not unlike those reported for non-halophites, there were significant differences between plants collected from the Texas City Dike area and those from Mud Island. Thus, shoot tissue of the Mud Island plants contained pentacosane (C_{25}) as the predominant hydrocarbon with a 3-fold difference in concentration over that of the Texas City Dike area plants. In addition, branched or unsaturated structural isomers of the *n*-alkanes were found only in the shoot tissue of the samples collected from Mud Island. Significant differences in the relative distribution of fatty acid constituents were noted in the shoots and roots from the two ecological areas. Shoot tissues from Mud Island contained saturated long-chain fatty acids (C_{20}, C_{22}) in high concentration whereas the shoot tissues from Texas City Dike plants contained the unsaturated C_{18} acids in high concentration. Root tissue fatty acids of both samples are more similar because of the high degree of saturation; there were however, concentration differences.

INTRODUCTION

Salicornia bigelovii is an obligate halophyte (salt plant) distributed throughout the world where areas of high salt concentration are prevalent.[1] Morphological and anatomical characteristics of halophytes such as succulence, reduced leaf size, lignified epidermal cell walls and a heavy cuticular waxy layer are similar to those of plants distributed in xerophytic environments.

Previous investigations have concentrated primarily on the taxonomy and ecology of the *Salicornia* species,[2] with little emphasis on chemical composition and physiology. Few studies have been directed toward correlating the physiological mechanisms with the high salt concentration of the cytoplasmic matrix.[2,3] Previous reports[4,5] indicate that enzymes and ribosomes which function in high salt concentrations have an unusual amino acid composition.

As previously mentioned, one of the important xerophytic anatomical characters associated with *S. bigelovii* is the epicuticular wax coating found on the shoot portions of the plant. The nature of the waxy coating in higher plants has been extensively reviewed by Eglinton and Hamilton.[6] The lipophilous components generally contained within this waxy layer are long-chain hydrocarbons, primary and secondary alcohols, ketones, esters, fatty

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[1] A. UNGAR, *Ecology* **47** (1) (1966).
[2] W. G. RIVERS, *Dissertation*, University of Houston (1969).
[3] M. INGRAM, in *Microbiol. Ecology*, Seventh Symp. Soc. Gen. Microbiol. (1957).
[4] S. T. BAYLEY and D. J. KUSHNER, *J. Mol. Biol.* **9**, 654 (1964).
[5] R. M. BAXTER and N. G. GIBBONS, *Can. J. Biochem. Physiol.* **32**, 206 (1954).
[6] G. EGLINTON and R. HAMILTON, *Science* **156**, 1322 (1967).

acids and acetals. The purpose of this investigation was to determine and compare the hydrocarbon and fatty acid distribution of *S. bigelovii* collected from two environments. The distribution of these compounds in the roots, shoots, and seeds of the respective plants were also compared.

RESULTS

Hydrocarbon analysis of the shoot tissues of *Salicornia bigelovii* collected from the Texas City Dike area reveal the presence of normal paraffinic alkanes ranging in carbon chain length from C_{21} to C_{33}. The compounds contained predominantly odd-numbered carbon chains; however, low concentrations of even-numbered carbon chain molecules were also detected. Molecules containing chain lengths of C_{27}, C_{29}, and C_{31} were found in greatest concentrations (Table 1). The odd-numbered carbon compounds increased in relative concentration with increasing chain length (Table 1).

TABLE 1. RELATIVE DISTRIBUTION OF HYDROCARBON COMPONENTS OF THE SHOOTS, ROOTS AND SEED OF *Salicornia bigelovii* COLLECTED FROM THE TEXAS CITY DIKE AREA AND MUD ISLAND

Hydrocarbon chain length	Texas City Dike area			Mud Island, shoot
	Shoot	Root	Seed	
C_{21}	9·5	10·1	—	trace
C_{22}	6·2	7·1	0·9	trace
C_{23}	2·9	10·6	2·8	1·3
C_{24}	—	16·1	2·6	1·8
C_{25}	11·0	25·2	6·7	30·0
C_{26}	4·6	9·2	4·7	3·1
C_{27}	15·2	11·5	9·7	9·2
iC_{27}	—	—	8·5	9·2
C_{28}	6·3	5·9	5·7	4·0
C_{29}	16·5	3·8	12·1	16·1
iC_{29}	—	—	12·8	10·7
C_{30}	5·1	—	4·9	—
C_{31}	17·5	—	9·0	4·4
iC_{31}	—	—	16·3	6·8
C_{32}	—	—	0·8	—
C_{33}	4·7	—	1·8	—

Root tissue analysis revealed a hydrocarbon distribution somewhat different from that of the shoot tissue. The *n*-alkane chain lengths ranged from C_{21} to C_{29} with no detectable quantities of C_{30}–C_{33} as found in the shoot. The even-numbered carbon alkanes were in highest quantities with C_{24} having the second largest relative concentration (Table 1). Pentacosane (C_{25}) was found in the greatest relative concentrations. There was a tendency for hydrocarbon components to increase in concentration with increasing chain length to C_{25}, with a decrease in concentration from C_{25} to C_{29} (Table 1).

The seed tissue contained a hydrocarbon distribution similar to that of the shoot tissue (C_{21}–C_{33}) with one notable exception: the predominant *n*-alkane components C_{27}, C_{29}, and C_{31} were found in almost equal concentrations with their respective structural isomers. The exact nature of these compounds is not known but their gas chromatographic behavior suggests they are branched or unsaturated paraffin hydrocarbons. The component designated

iC_{31} was found in the greatest concentrations with C_{29} and iC_{29} in the second largest quantities. The relative distributions of the hydrocarbon extracts of seed tissue appears to have a more even distribution of the alkane components with high concentrations of the even-numbered carbon components (Table 1).

The shoot tissue lipid extracts of samples collected from Mud Island contained hydrocarbon components ranging in carbon chain lengths from C_{21} to C_{31}. In addition, structural isomers of C_{27}, C_{29}, and C_{31} were also detected in relatively high concentrations (Table 1). Pentacosane was found in the highest relative concentrations at 30·0 per cent followed by C_{29} at 16·1 per cent (Table 1). Again compounds containing even-numbered carbon chains were found in low relative concentrations (Table 1).

Analysis of the saponifiable lipid extracts from the same *S. bigelovii* samples revealed the presence of saturated and unsaturated fatty acids ranging in chain length from C_{14} to C_{24}. The predominant fatty acids contained hydrocarbon chains with an even number of carbon atoms while the odd-numbered carbon chains were in low relative concentrations.

The total fatty acid distribution of the shoot, root, and seed tissues collected from the Texas City Dike area revealed great variability. The predominant saturated acid of the shoot and root was palmitic, whereas stearic was the major acid of the seed tissues. No similarities were noted in the distribution of the major unsaturated ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$) acids of the three tissues. Shoot tissue contained predominant concentrations of linolenic acid ($C_{18:3}$); root tissue contained almost equal concentrations of oleic and linoleic acids ($C_{18:1}$ and $C_{18:2}$ respectively); while the seed tissues contained almost exclusively oleic acid (Table 2). In all cases the saturated to unsaturated ratio was less than one.

TABLE 2. RELATIVE DISTRIBUTION OF THE TOTAL FATTY ACID EXTRACTS OF THE ROOTS, SHOOTS AND SEEDS OF *Salicornia bigelovii* COLLECTED FROM THE TEXAS CITY DIKE AREA AND MUD ISLAND

Hydrocarbon chain length	Texas City Dike area				Mud Island	
	Shoot	Root	Seed	Seed†	Shoot	Root
C_{14}	1·9	1·9	trace	0·6	2·8	1·5
$C_{14:1}$	—	1·1	9·1	7·9	—	—
C_{16}	12·0	19·1	trace	1·7	17·3	9·4
$C_{16:1}$	trace	4·2	trace	—	—	2·6
C_{17}	—	1·1	2·9	1·5	3·0	—
C_{18}	1·1	5·3	13·6	22·8	1·6	1·8
$C_{18:1}$	14·5	24·7	67·8	48·6	3·7	13·8
$C_{18:2}$	21·5	28·7	3·6	9·6	15·2	49·7
$C_{18:3}$*	47·9	7·7	1·8	—	1·8	4·1
C_{20}	—	—	—	—	22·4	1·6
C_{22}	0·8	6·1	6·1	—	28·3	4·3
C_{24}	—	—	—	—	3·3	—

* Values recorded for $C_{18:3}$ include those for C_{20} of the Texas City Dike samples. The two compounds were not resolved with the EGS column used in this study. Later studies reveal the presence of small concentrations of C_{20} and C_{24} in these samples.

† Triglyceride hydrolysate.

Triglycerides represented 75–85 per cent of the total lipid extracts from seed tissue. Triglyceride hydrolysates revealed that stearic and oleic acids were found in an approximate 1:2 ratio whereas other fatty acids were found in low relative concentrations (Table 2).

The shoot and root total fatty acids extracted from samples collected from Mud Island revealed an altogether different distribution. Shoot fatty acids contained a saturated to unsaturated ratio greater than one, whereas the reverse was true for the root fatty acids. The predominant shoot fatty acids were palmitic, arachidic, and behenic. Palmitic was the predominant saturated acid from the root tissues but in low relative concentrations. Linoleic was the predominant unsaturated acid for both tissues of the Mud Island samples but the root tissue contained a 3·3-fold larger concentration (Table 2).

DISCUSSION

The paraffin hydrocarbon and fatty acid distributions of *Salicornia bigelovii* conform closely to those reported for other higher plants and fungal spores.[7-11] It appears that the specific adaptation to the halophilic environment is expressed not as a change in the enzymes of lipid metabolism but rather in the degree of tolerance or adaptation of the enzymes to the high salt concentrations found within the cytoplasmic matrix.

Hydrocarbon and fatty acid analyses of samples collected from the Texas City Dike area further illustrate how distributions of chemical constituents vary from tissue to tissue within a single plant.[11] Thus, there are very different distributions of hydrocarbons and fatty acids in the shoots, roots, and seeds of *S. bigelovii.*

Specific external environments as well as different cellular (ionic) environments probably are the major factors influencing these observed differences. For example, it is reported that as much as 2-fold differences in the Na^+, K^+, Mg^{2+}, and Cl^- concentrations exist between the root, shoot and seeds of *S. bigelovii* collected from both the Texas City Dike area and Mud Island.[2] However, significant changes due to seasonal influences were observed. The same ion differences may exist in glycophilic plants but not at such high concentration levels.

Solubility factors probably play an important role in the synthesis of lipophilic molecules containing a hydrophobic hydrocarbon chain. Because of the polar nature of aqueous solutions (assumed cytoplasmic environment of halophytes) of high ionic strength there is a possibility that isolating mechanisms may be operative in the cell giving rise to relatively non-polar areas where synthesis of long hydrocarbon chains may occur.

Drastic differences in total fatty acid distribution were also noted for the roots and shoots of *S. bigelovii* collected from Mud Island. There is a dramatic difference in the degree of saturation between these two tissues. The highly unsaturated root fatty acids may be explained on the basis of temperature and physical state (solid or liquid) relationships. The water-submerged roots would obviously have cooler temperatures requiring a higher degree of unsaturation required to maintain the liquid or soluble state. Higher unsaturation levels probably exist because of the increased availability of O_2 due to its greater solubility at lower temperatures. On the other hand, differences in ion content were also found between these tissues.[2] Thus, the ionic or polar differences found in different plant tissues may result in alterations in the synthesis of the hydrocarbon and fatty acid components of halophytic plants.

The presence of structural isomers in the shoot tissues of Mud Island samples were similar to those in the seed tissue of plants from the Texas City Dike area. There may be two primary

[7] P. F. WILDE and P. S. STEWART, *Biochem. J.* **102**, 225 (1968).
[8] J. D. WEETE, J. L. LASETER, D. J. WEBER, W. M. HESS and D. J. STOCKS, *Phytopathology* **59**, 545 (1969).
[9] J. L. LASETER, J. WEETE and D. J. WEBER, *Phytochem.* **7**, 1177 (1968).
[10] J. L. LASETER, W. M. HESS, J. D. WEETE, D. J. STOCKS and D. J. WEBER, *Can. J. Microbiol.* **14**, 1149 (1968).
[11] F. B. SHORLAND, *Comparative Biochemistry*, Academic Press, New York (1962).

reasons for these isomeric components to be specifically located in the seed tissue. First, the fertile seed-bearing tissues located at the stem tips may be the only source of proper substrates, or secondly, these are the only tissues where the specific enzymes for their synthesis are active or present. The latter seems to be the most plausible explanation on the basis of recent studies involving localization of hydrocarbon and very long chain fatty acid (C_{24}–C_{26}) synthesis in leaf tissue. It has been demonstrated that enzymes necessary for the synthesis of these compounds could be isolated in a specific tissue (epidermis of leaf) and that the lack of branched-chain hydrocarbons was not due to the lack of suitable substrates.[12–14] Thus, it seems that the appearance of these structural isomers is a function of the degree of development of the fertile tissue at the stem tip.

Again, dramatic differences were noted in the total fatty acids between the roots and shoots of samples collected from both areas. The explanation for these differences are probably the same as those for the differences in paraffin distribution.

It seems obvious that high salt concentrations have a dramatic influence on the metabolic processes of obligate halophytic plants. There is one primary question that remains unanswered: at what level of synthesis does the ionic influence occur? From the results presented here, it can be concluded that high ionic concentrations of *S. bigelovii* do not qualitatively affect the ultimate hydrocarbon and fatty acid content. It appears that differences in ion concentration influence the relative proportion of these individual hydrocarbon and fatty acid components but the range of these components is similar to those found in non-halophytic plants. This suggests that the enzymes have capabilities of adapting to the high ionic concentrations and that the level of ionic influence appears to be located at some other synthetic level.

EXPERIMENTAL

Samples of *Salicornia bigelovii* used for analysis in this study were selected from the Texas City Dike area and Mud Island off the Texas Gulf Coast.

Samples of *S. bigelovii* were collected from the Texas coastal salt marsh. The sites of collection were located on the Texas City Dike, Galveston County, Texas, and Mud Island, near San Luis Pass, Brazoria County, Texas., U.S.A. At high tide, at the Mud Island site, the sea covered the stems of the plants to a height of approximately 20 cm. At the Texas City Dike site the plants were not reached by sea-water.

The plants were immediately frozen and stored at −4°. The frozen plants were divided into roots and shoots and then lyophilyzed for 24 hr prior to extraction. Two to five grams (dry wt.) of material were extracted with 100 ml benzene:$CHCl_3$ (3:1) overnight at room temperature with constant agitation. After decanting, the same material was extracted for several hours with *n*-heptane under the same conditions. The combined extracts were taken to dryness under N_2. The total lipid extract was subjected to alkaline hydrolysis by the method of Wilde and Stewart.[7] The total fatty acids were analyzed as their methyl esters as previously described.[8] The non-saponifiable material was further purified for hydrocarbon components by placing it on a pre-treated (200°, 1 hr) silica gel column, eluting with *n*-heptane and taking to dryness as before. The natural hydrocarbon components were injected directly into a Perkin–Elmer 900 gas chromatograph equipped with a 50 ft × 0·03 in. stainless-steel capillary tubing coated with OV-1 or SE-30. The fatty acid methyl esters were separated on a 10 ft × ⅛ in. stainless-steel tubing packed with Chromosorb W coated with 15% ethylene glycol succinate or diethylene glycol succinate.

Seed tissue triglycerides were separated from the total lipid extract by TLC on silica gel in heptane–Et_2O–HOAc (85:20:2). The triglyceride components were identified by co-chromatography with standards. The triglycerides were isolated from the silica gel by eluting with benzene in a glass column (3 × 1·5 cm) and then the triglycerides were hydrolyzed.

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[12] P. E. Kolattukudy, *Plant Physiol.* **43**, 375 (1968).
[13] P. E. Kolattukudy, *Plant Physiol.* **43**, 1466 (1968).
[14] P. E. Kolattukudy, *Plant Physiol.* **43**, 1423 (1968).