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HYDROCARBONS AND WAX ESTERS FROM SEVEN SPECIES OF MANGROVE LEAVES

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Key Word Index—*Avicennia officinalis*; Avicenniaceae; *Acanthus illicifolius*; Acanthaceae; *Bruguiera gymnorhiza*; Rhizophoraceae; *Ceriops decandra*; Rhizophoraceae; *Derris trifoliata*; Fabaceae; *Rhizophora mucronata*; Rhizophoraceae; *Suaeda maritima*; Chenopodiaceae; hydrocarbons; wax esters.

Abstract—Seven species of fresh mangrove leaves were found to contain saturated normal and branched chain hydrocarbons, mostly between C_{16} and C_{36} with both odd and even carbon numbers. Significant quantitative variations were found between species. Wax esters were found to contain fatty acids with chain lengths between C_{12} and C_{22}. Palmitic (16:0) and stearic (18:0) acids were the major component saturated fatty acids, whereas, oleic (18:1) and linolenic (18:3) acids were the major unsaturate α-acids. Chain lengths of the alcohols of wax esters were between C_{14} and C_{36}. Significant quantitative and minor qualitative differences were noted in the alcohol composition of wax esters. Hydrocarbon and wax ester compositions were characterised by the presence of low M_r components in high proportions.

INTRODUCTION

In plants, the cuticle surface which is exposed to the atmosphere usually has a layer of wax, composed of mainly wax esters and hydrocarbons [1]. Stem, fruits, petals and leaves, may all be covered with wax, though leaf waxes have received most attention. The wax when present, undoubtedly serves to preserve the water balance of the plant and may vary considerably under abnormal conditions as indicated in ref. [2].

The alkanes of cuticular waxes may be saturated, unsaturated or branched and may have various chain lengths. Wax esters contain fatty acids and alcohols of various chain lengths. Considerable work on the epicuticular waxes have been done by Tulloch *et al.* [3–10]. In the present study the compositions of hydrocarbons and fatty acids and alcohols of wax esters of the leaves of seven species of mangrove plants have been determined.

RESULTS AND DISCUSSION

The various lipid contents of the fresh leaves from the seven species of mangrove plants are presented in Table 1. The total lipids, hydrocarbons, wax esters and sterols were present in highest proportion in *Derris tritoliata*, whereas, the highest proportions of sterol esters and triacylglycerols were present in *Avicenna officinalis*. Of the other constituents, polar lipid was predominant in *Bruguiera gymnorhiza*; terpenes were present in the highest proportion in Rhizophora mucronata.

Hydrocarbon compositions (Table 2), were mostly between C_{16} and C_{36}, distributed evenly with no component exceeding 16%. *Acanthus illicifolius*, *Suaeda maritima* and *Ceriops decandra* also contained low levels of hydrocarbons with chain lengths of C_{37} and C_{38}. Among the branched chain hydrocarbons, only the *anteiso*-series was present with chain lengths of C_{27}–C_{29}, C_{31} and C_{33}. Alkenes were absent and only saturated normal and branched hydrocarbons were present, of which mostly even carbon chain hydrocarbons were major components. Hydrocarbons in these leaves were characterised by the predominance of short carbon chain lengths. Similar findings were reported in the pneumatophores of *Avecennia marina* [11], and also in our previous study [12]. Qualitative compositions were similar with minor variations, whereas, significant quantitative variations were observed between the samples.

The fatty acid compositions of the wax esters (Table 3), indicates that, the carbon chain lengths of the various fatty acids ranged from C_{12} to C_{22} with 16:0, 18:0, 18:1ω9, 18:2ω6 and 18:3ω3, as the predominant acids. These data were similar to those reported previously for *A. officinalis*, *A. illicifolius* and *Briguiera gymnorhiza* [12–14]. Among the fatty acids, 16:0 was the major component in all samples. Of the unsaturated fatty acids, 18:1ω9 was the major component in *A. officinalis*, *B. gymnorhiza* and *A. illicifolius*, whereas, 18:3ω3 was the major component in the rest of the species. The highest proportion of 18:3ω3 was found in *C. decandra* whereas it was absent in *A. officinalis*. 18:2ω6 was present in variable amounts, ranging from 2% in *A. illicifolius* to as much as 16.5% in *B. gymnorhiza*. Of the other unsaturated fatty acids, 16:1 was present as a minor component in four species and 22:1 in five. The total saturated fatty acids were mostly over 50%, the highest proportion being 80.5% in *R. mucronata*. On the other hand, the total unsaturated fatty acids of *A. officinalis* and *C. decandra* were over 50% of the total fatty acids.

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Table 1. Lipid contents* of seven species of mangrove leaves

Lipid components	Species 1†	2	3	4	5	6	7
Total lipid	15000	13600	6200	6400	3500	15300	25000
Hydrocarbon	765	700	470	700	500	2000	3500
Wax ester	640	500	780	1000	400	1900	3400
Steryl ester	4345	1400	1406	1100	500	2000	3600
Triglyceride	5520	1350	2528	1100	600	3000	4500
Sterol	1000	1200	450	1200	600	2800	5300
Polar lipid	2730	8450	566	1300	900	3600	4700
Terpene alcohol	900	1300	2400	8100	2000	7700	3100
Terpene acid	700	1600	3700	8300	—	3400	—

*Expressed as μg/g of fresh leaves; terpenes are not included in total lipids.

†1. *Avicennia officinalis*; 2. *Bruguiera gymnorhiza*; 3. *Acanthus illicifolius*; 4. *Rhizophora mucronata*; 5. *Suaeda maritima*; 6. *Ceriops decandra*; 7. *Derris trifoliata*.

Table 2. Hydrocarbon composition (% total) of seven species of mangrove leaves

Carnon number	Species 1*	2	3	4	5	6	7
16:0	7.8	4.0	6.0	0.5	3.5	2.7	3.3
18:0	7.6	10.9	12.5	2.5	2.6	4.0	3.0
19:0	0.4	—	—	1.3	—	—	—
20:0	5.0	3.5	9.2	11.8	9.0	10.8	6.2
21:0	0.3	2.0	1.0	0.6	0.6	1.0	0.2
22:0	2.3	8.5	4.0	5.3	8.4	5.0	7.8
23:0	1.0	8.5	2.0	0.8	9.0	3.9	2.8
24-*anteiso*	—	—	1.0	—	—	—	—
24:0	2.4	9.2	4.5	6.0	9.0	11.3	7.9
25:0	3.3	3.5	2.5	4.9	12.0	7.2	4.8
26:0	3.2	4.0	2.5	3.9	5.6	6.8	5.4
27-*anteiso*	0.3	2.2	1.0	1.3	4.7	2.7	2.2
27:0	6.0	16.1	2.0	5.4	12.0	5.8	6.5
28-*anteiso*	0.1	1.0	0.5	0.8	1.2	1.2	0.6
28:0	2.2	6.5	2.0	3.8	2.4	7.0	2.0
29-*anteiso*	2.2	2.5	0.8	0.9	2.2	1.1	0.9
29:0	6.3	11.5	3.0	9.2	4.1	6.3	7.6
30:0	9.9	1.0	4.0	6.0	2.2	6.2	9.9
31-*anteiso*	6.0	0.7	—	—	1.2	—	—
31:0	10.8	1.2	8.0	16.1	2.2	9.8	11.6
32:0	6.5	1.2	8.5	7.0	2.2	2.4	5.8
33-*anteiso*	3.6	—	—	4.5	1.4	—	—
33:0	10.6	—	12.0	5.7	1.6	2.2	6.5
34:0	0.7	0.6	4.0	0.5	0.4	0.7	2.0
35:0	0.7	0.5	6.0	0.8	0.9	0.5	1.2
36:0	0.8	0.9	1.0	0.4	0.4	0.5	1.8
37:0	—	—	1.5	—	0.3	0.4	—
38:0	—	—	0.5	—	0.9	0.5	—

*See Table 1 for footnotes.

Table 3. Fatty acid compositions (% total) of the wax esters of seven species of mangrove leave

Carbon number	Species 1*	2	3	4	5	6	7
12:0	2.5	2.0	5.5	1.1	3.5	5.3	6.3
14:0	2.8	5.0	4.8	2.7	8.0	6.5	7.3
16:0	40.0	44.0	48.0	56.6	28.8	26.2	26.0
16:1	—	—	—	3.8	7.2	2.5	5.5
17:0	—	—	—	—	3.2	2.1	1.9
18:0	4.0	10.0	17.5	17.5	10.6	6.9	16.0
18:1	36.0	19.0	15.2	6.2	11.3	12.7	5.9
18:2	14.2	16.5	2.0	2.7	5.0	10.2	8.3
18:3	—	1.5	1.0	6.8	21.0	24.9	18.5
20:0	0.5	2.0	—	1.6	0.3	—	1.7
22:1	—	—	6.0	1.0	1.1	2.7	2.6

*See Table 1 for footnotes.

The distribution of alcohols in seven mangrove leaves is typical of plants of the mangrove environment as published earlier [12, 13], with even carbon chains predominating over odd. The proportion of C_{24} was highest in all the species except *D. trifoliata* and *A. illicifolius*, where, C_{32} and C_{20} respectively, were the major components. The longest chain length found was C_{36}, present in all samples except *A. officinalis*. Of the unsaturated alcohols, C_{14} was present in all species except *B. gymnorhiza*, whereas, C_{18} was present only in *B. gymnorhiza* and C_{26} only in *A. officinalis*. Significant qualitative and quantitative variations in alcohol compositions were observed among the samples (Table 4).

Ester profiles (Table 5), ranged from C_{26} to C_{50} with C_{30}, C_{32}, C_{36}, C_{38}, C_{40}, C_{42}, as the predominant components in almost all the leaves. Ester components of higher carbon chain lengths, such as C_{44} and C_{46}, were present in appreciable amounts in *B. gymnorhiza* and *D. trifoliata*; C_{46}, C_{48} and C_{50} components were present in significant quantities in *D. trifoliata*. The homologue distribution of the constituent fatty acid and alcohol fractions were related to the corresponding ester profiles. Fatty acids of the wax esters were principally C_{16} and C_{18} and the presence of high proportions of short chain ester alcohols provided large ester peaks in the range of C_{30}–C_{42}. The presence of significant quantity of longer chain length homologues of esters in *B. gymnorhiza* and *D. trifoliata* is probably because of the presence of appreciable amounts of the long chain alcohols C_{32} and C_{34} in these two plants.

Table 4. Alcohol composition (% total) of wax esters of seven species of mangrove plant leaves

Carbon number	Species 1*	2	3	4	5	6	7
14:1	0.5	—	8.5	11.3	6.8	9.5	3.9
14:0	0.6	—	10.0	12.3	14.3	10.1	6.6
16:0	1.0	2.8	6.0	12.3	3.2	4.7	—
17:1	1.5	1.0	1.0	—	—	—	—
17:0	1.6	3.0	1.0	3.7	0.8	1.9	—
18:1	—	0.5	—	—	—	—	—
18:0	2.0	1.0	6.2	10.3	6.3	5.0	8.6
20:1	1.5	—	1.0	—	1.2	1.0	0.4
20:0	3.0	3.0	12.6	3.0	9.2	7.6	1.5
21:1	0.8	—	—	—	—	—	—
21:0	5.0	—	2.5	—	4.1	2.8	0.4
22:1	0.3	—	1.5	—	—	—	—
22:0	2.6	13.0	6.8	4.1	6.2	7.1	1.3
23:1	0.2	1.0	—	—	—	—	—
23:0	2.0	5.0	—	—	—	—	2.0
24:0	48.7	30.0	9.1	21.0	29.5	25.2	4.6
25:0	0.7	—	—	—	—	—	—
26:1	0.4	—	—	—	—	—	—
26:0	8.0	9.0	2.5	2.5	3.2	1.9	3.5
27:0	0.8	1.5	2.6	—	—	—	—
28:1	1.0	2.0	—	—	—	—	1.5
28:0	6.5	7.0	5.5	6.2	4.1	9.1	4.3
29:0	0.5	—	—	—	—	—	6.1
30:0	4.2	6.0	5.5	3.0	2.0	1.9	2.4
31:0	0.1	—	—	—	—	—	4.0
32:0	4.0	9.2	2.2	3.7	3.6	3.8	29.0
34:0	2.5	2.5	9.5	3.0	2.4	3.0	18.3
36:0	—	2.5	8.0	3.6	3.1	6.3	1.6

*See Table 1 for footnotes.

Table 5. Wax ester compositions (% total) of seven species of mangrove plant leaves

Carbon number	Species 1*	2	3	4	5	6	7
26	—	—	2.0	0.6	1.5	2.0	0.5
28	—	—	1.0	0.6	3.0	3.5	1.0
29	0.5	1.0	0.5	—	—	0.5	0.5
30	0.5	—	21.0	18.0	15.0	12.0	7.0
31	1.0	0.5	1.0	0.5	—	1.0	—
32	1.0	0.5	18.0	15.0	17.0	21.0	9.0
33	1.5	—	1.0	2.0	—	1.0	—
34	1.0	5.0	2.5	10.0	6.0	3.0	5.0
35	1.0	6.0	0.5	1.0	0.5	—	0.5
36	9.0	8.0	13.0	8.5	5.0	3.5	6.0
37	5.7	0.5	1.0	—	2.5	—	0.5
38	10.2	15.0	12.0	2.5	10.0	5.5	1.0
39	9.2	0.5	0.6	—	5.5	—	1.0
40	25.0	20.0	8.0	19.0	15.0	13.0	3.5
41	—	—	—	—	—	—	1.5
42	28.0	29.0	8.0	12.5	17.0	16.0	2.7
43	—	—	—	—	—	—	2.5
44	5.5	9.0	3.0	7.0	1.0	10.0	10.5
45	—	—	—	—	—	—	—
46	1.0	5.0	5.0	2.8	1.0	8.0	12.3
48	—	—	1.0	—	—	—	16.0
50	—	—	1.0	—	—	—	19.0

*See Table 1 for footnotes.

In the present study, the hydrocarbon and wax ester profiles of the mangrove plant leaves were characteristic in the sense that, both contained a high proportion of low M_r components.

EXPERIMENTAL

Plant material. Leaf samples were collected from Prentice Island, between latitudes 21.43 and 21.46°N and longitudes 88.18 and 88.19°E of the Sunderbans mangrove forest, West Bengal, India. Leaves were washed thoroughly with dist. H_2O before analysis.

Isolation and fractionation of total lipids. Leaves were cut into pieces and lipids were extd as described in ref. [15]. Lipid classes were separated by prep. TLC [6]. Hydrocarbon and wax ester bands which were not resolved were further sepd by prep. TLC using Et_2O–*n*-hexane (1:49). Hydrocarbon and ester fractions were recovered from the plates and weighed.

Analysis of hydrocarbons. Hydrocarbons were analysed directly by GC according to ref. [17].

Analysis of wax esters. Esters were hydrolysed by pancreatic lipase on TLC plates and the resulting fatty acids and alcohols analysed as described in ref. [13]. GC of esters was carried out on a 3% SE-30 column (1.8 m × 3 mm). Oven temp. was initially isothermal at 230° for 16 min and then programmed (4° min) up to 350°. Peaks were identified by addition of authentic compounds, synthesized as in ref. [13].

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