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EFFECT ON THE LEAF LIPIDS OF THREE SPECIES OF MANGROVE OF PERIODIC SUBMERGENCE IN TIDAL WATER

SUNITI MISRA, A. CHOUDHURY, PRASANTA K. PAL* and AMITABHA GHOSH*†

Marine and Estuarine Biological Research Unit, Department of Marine Science, University of Calcutta, 35, B.C. Road, Calcutta 700019, India; *Department of Chemistry, Bose Institute, 93/1 A.P.C. Road, Calcutta 700009, Inida

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Key Word Index -*Acanthus illicifolius*; Acanthaceae; *Avicennia officinalis*; Avicenniaceae; *Bruguiera gymnorhiza*; Rhizophoraceae; water stress; lipids; terpenoids.

Abstract—Mangrove plant leaves of *Avicennia officinalis*, *Acanthus illicifolius* and *Bruguiera gymnorhiza*, grown under tidal water stress, were found to biosynthesize higher proportions of hydrocarbons, wax esters, sterol esters, triglycerides, sterols and low-M_r terpenes, compared to the plants grown normally. Hydrocarbons and *n*-alcohols of wax esters, with longer chain lengths were found in higher proportions in the plants grown normally. Higher proportions of unsaturated *n*-alcohols of wax esters were present in the leaves of periodically submerged plants. Proportions of unsaturated fatty acids of wax esters, sterol esters and triglycerides were also higher in the submerged plants. Sitosterol, stigmasterol and campesterol of the sterol esters, were higher in submerged plants, whereas 28-isofucosterol was higher in normal plants. Of the free sterols, sitosterol was higher in submerged plants, whereas stigmast-7-en-3β-ol and campesterol were higher in normal plants and 28-isofucosterol was found only in submerged plants. Pentacyclic triterpenoids were found only in normal plants, whereas, the submerged plants contained low-M_r terpenes in higher proportions.

INTRODUCTION

Mangrove forests are important components of subtropical and tropical estuarine ecosystems, and form a natural transition between the land and the sea. Some mangrove plants which grow near the banks are completely submerged at high tide, twice each day, others grow normally on the deltaic ridges and are not submerged under tidal water. It was observed by Misra *et al.* [1] that accumulation of higher proportions of hydrocarbons and wax esters in the leaves of the periodically submerged species make them tolerent to periodic flooding. In the present communication the compositions of the lipid components in the leaves of three species of mangrove plants grown normally and under tidal water stress have been compared.

RESULTS AND DISCUSSSION

The total lipid, hydrocarbon, wax ester, steryl ester, triglyceride, sterol and terpene contents of three species of plants (*Avicennia officinalis* L., *Acanthus illicifolius* L., *Bruguiera gymnorhiza* Lam.) grown normally and in periodically submerged condition under tidal water are given in Table 1. The total lipids were present in higher proportions in submerged plant leaves, except in *B. gymnorhiza*. Hydrocarbons and wax esters in the submerged plants were about three times greater than those in normal plants, as was observed previously [1]. The other constituents, i.e. steryl esters, triglycerides, sterols and terpenes were also present in higher proportions in the submerged plants.

The hydrocarbon compositions of normal and submerged plants are shown in Table 2. It was found that the shorter chain hydrocarbons were present in higher proportions in the submerged plants. Some qualitative differences were also observed.

The alcohol compositions of the normal and submerged plants are shown in Table 3. The *n*-alcohols both saturated and unsaturated, with shorter chain lengths were present in higher proportions in the submerged plants. Some qualitative differences were also observed. The fatty acid compositions of the wax esters (Table 4) indicated that higher proportions of unsaturated fatty acids were present in submerged plants.

The fatty acid compositions of the steryl esters (Table 5) indicated that higher proportions of unsaturated fatty acids were present in the submerged plants. It is interesting to note that the proportions of oleic acid (18:1) were higher in the normal plants of the three species. Regarding qualitative differences, longer chain fatty acids, i.e. C_{20} and C_{22}, were only present in submerged plants. The sterol compositions of the steryl esters (Table 6) indicated that sitosterol, stigmasterol and campesterol were higher in submerged plants, whereas cholesterol and 28-isofucosterol were higher in normal plants. Stigmast-7-en-3β-ol was present only in submerged plants.

The fatty acid compositions of triglycerides are shown in Table 7. The major component fatty acids were 16:0, 18:1, 18:2 and 18:3. Of the unsaturated acids, only 18:2 was present in higher proportions in normal plants. The proportions of total unsaturated acids were always higher in submerged plants.

The compositions of the free sterols are shown in Table 8. Sitosterol was present in higher proportions in submerged plants, whereas all other sterols were higher in

†To whom correspondence should be addressed.

Table 1. Compositions (μg/g fr.wt. of leaves) of total lipids and other lipid components of three species of leaves grown normally and under tidal water

Lipid component	*A. officinalis*		*A. illicifolius*		*B. gymnorhiza*	
	S*	N*	S	N	S	N
Total lipids	30750	14100	12565	6000	9374	13200
Hydrocarbons	2820	711	1330	440	1803	607
Wax esters	2051	646	1848	800	1020	462
Steryl esters	13991	4399	3895	1416	1656	1554
Triglycerides	6150	5724	2424	2328	1400	1344
Sterols	3790	1000	770	320	1200	1000
Terpenes	2366	1600	6342	6100	3386	3000

*S, Submerged under tidal water; N, normal.

Table 2. Hydrocarbon compositions (% total) of three species of mangrove leaves grown normally and under tidal water

component	*A. officinalis*		*A. illicifolius*		*B. gymnorhiza*	
	S*	N*	S	N	S	N
16:0	6.7	4.8	11.2	6.9	16.7	4.7
17:0	—	—	—	—	10.3	—
18:0	12.1	5.0	23.3	13.0	16.7	11.9
19:0	—	0.4	—	—	1.6	—
20:0	3.8	5.0	15.0	8.6	12.3	3.5
21:0	—	0.3	—	1.1	1.1	2.4
22:0	2.7	2.2	9.0	4.0	6.5	7.5
23:0	—	0.9	—	1.9	—	7.5
24-anteiso	—	—	—	1.0	—	—
24-iso	0.4	—	—	—	—	—
24:0	2.0	2.4	9.6	3.9	4.0	8.6
25:0	3.3	3.3	—	3.0	2.7	3.9
26-anteiso	4.6	—	—	—	3.0	—
26-iso	—	—	—	—	0.4	—
26:0	14.6	3.2	1.2	2.8	7.4	4.5
27-anteiso	—	0.3	—	0.8	—	2.5
27:0	3.4	6.0	—	2.7	2.7	14.9
28-anteiso	—	0.1	—	0.5	—	1.6
28-iso	0.8	—	—	—	—	—
28:0	15.4	2.2	2.3	2.5	9.7	7.0
29-anteiso	—	2.2	—	0.9	—	2.5
29:0	2.8	6.3	—	2.7	0.7	10.8
30-iso	0.2	—	—	—	—	—
30:0	16.6	9.9	6.4	3.8	1.4	1.0
31-anteiso	—	6.0	—	—	—	0.5
31:0	1.1	16.6	3.9	7.6	0.8	1.5
32:0	5.3	6.5	11.0	8.3	0.8	1.2
33-anteiso	—	3.6	—	—	—	—
33:0	—	10.6	0.8	10.8	0.5	—
34:0	1.6	0.7	2.3	4.2	0.7	0.7
35:0	—	0.8	—	5.0	—	0.5
36:0	2.6	0.8	—	1.4	—	0.8
37:0	—	—	—	1.9	—	—
38:0	—	—	—	0.7	—	—

*S, Submerged under tidal water; N, normal.

normal plants. An unidentified component and 28-isofucosterol were present only in submerged plants. A comparison of the data in Tables 6 and 8 reveals that esterified 28-isofucosterol was present both in normal and submerged plants, whereas the free sterol was only present in submerged plants. On the other hand, unesterified

Table 3. Alcohol compositions (% total) of the wax esters of three species of mangrove leaves grown normally and under tidal water

Components	*A. officinalis*		*A. illicifolius*		*B. gymnorhiza*	
	S*	N*	S	N	S	N
14:U	4.0	0.6	11.8	9.0	—	—
14:0	5.0	0.6	12.0	9.8	—	—
16:0	2.1	1.0	12.2	5.7	4.0	3.0
17-U	2.5	1.7	1.7	0.9	1.5	0.8
17:0	2.5	1.8	2.0	1.0	3.5	3.0
18-U	—	—	—	—	2.0	0.5
18:0	3.1	2.2	10.0	6.5	3.5	0.9
20-U	1.8	1.6	1.0	1.0	0.5	—
20:0	5.0	3.1	14.8	12.5	6.0	3.0
21-U	1.2	0.8	—	—	—	—
21:0	7.0	4.9	0.8	2.8	3.0	—
22:U	0.6	0.3	1.0	1.5	—	—
22:0	5.5	2.5	4.2	6.5	15.5	12.7
23-U	1.0	0.3	—	—	1.5	1.0
23:0	4.5	2.2	—	—	5.0	5.0
24:0	39.0	48.0	7.4	9.0	26.5	31.0
25:0	1.0	0.8	—	—	—	—
26-U	1.0	0.5	—	—	—	—
26:0	6.4	8.3	1.0	2.8	6.5	8.8
27:0	0.5	0.8	2.4	2.8	1.5	1.5
28-U	0.5	1.0	—	—	2.0	2.0
28:0	4.5	6.3	3.5	5.0	5.0	6.5
29:0	0.5	0.5	—	—	—	—
30:0	0.8	3.8	4.2	5.0	5.0	5.8
31:0	—	0.1	—	—	—	—
32:0	—	4.0	1.5	1.8	7.5	9.0
34:0	—	2.3	5.5	9.0	—	3.0
36:0	—	—	3.0	7.4	—	2.5

*S, Submerged under tidal water; N, normal.

Table 4. Fatty acid compositions (% total) of wax esters of three species of mangrove leaves grown normally and under tidal water

Component	*A. officinalis*		*A. illicifolius*		*B. gymnorhiza*	
	S*	N*	S	N	S	N
12:0	0.8	2.0	3.7	5.3	1.5	2.5
14:0	1.0	2.5	4.0	4.2	3.0	4.5
16:0	37.5	39.1	40.8	46.5	40.0	42.0
18:0	4.8	4.5	20.5	17.5	9.0	10.5
18:1	38.3	36.5	20.1	16.5	21.8	19.7
18:2	15.8	14.9	3.7	2.5	19.5	17.5
18:3	—	—	1.8	1.0	3.5	1.3
20:0	1.8	0.5	—	—	1.7	2.0
22:1	—	—	5.4	6.5	—	—

*S, Submerged under tidal water; N, normal.

Table 5. Fatty acid compositions (% total) of the steryl esters of three species of mangrove leaves grown normally and under tidal water

Components	*A. officinalis*		*A. illicifolius*		*B. gymnorhiza*	
	S*	N*	S	N	S	N
16:0	14.0	30.8	12.0	18.2	12.9	24.8
18:0	8.0	18.9	4.5	7.8	6.0	8.0
18:1	16.0	24.0	14.0	22.0	13.0	18.0
18:2	33.0	18.0	35.5	30.0	36.0	30.0
18:3	22.0	9.5	28.0	22.0	25.0	19.2
20:0	1.0	—	0.5	—	1.0	—
22:0	1.0	—	0.5	—	0.5	—
22:1	5.0	—	5.0	—	5.6	—

*S, Submerged under tidal water; N, normal.

Table 6. Sterol compositions (% total) of steryl esters of three species of mangrove leaves grown normally and under tidal water

Compound	*A. officinalis* S*	*A. officinalis* N*	*A. illicifolius* S	*A. illicifolius* N	*B. gymnorhiza* S	*B. gymnorhiza* N
Unidentified	2.6	—	0.7	—	1.0	—
Cholesterol	4.3	8.0	5.4	7.0	4.0	4.6
Campesterol	4.7	1.3	16.0	14.0	8.3	6.0
Stigmasterol	12.0	2.9	16.0	11.0	9.4	7.7
Sitosterol	69.6	50.0	60.4	54.0	70.1	61.8
28-Isofucosterol	4.9	37.8	1.0	14.0	3.5	12.8
Stigmast-7-en-3β-ol	1.9	—	0.5	—	0.8	—
Unidentified	—	—	—	—	1.0	7.0

*S, Submerged under tidal water; N, normal.

stigmast-7-en-3β-ol was present both in normal and submerged plants, whereas the esterified form was only present in normal plants.

The pentacyclic triterpenoids found in the normal plants were, α-amyrin, β-amyrin, lupeol, oleanolic acid and ursolic acid, in proportions similar to those reported earlier [2]. GLC analysis of the terpenes of submerged plants revealed the presence of components with retention times lower than those of pentacyclic triterpenoids. As the separation of GLC is primarily based on M_rs, the lower retention times of the terpenes indicate that, in the submerged plants, the M_rs of the terpenes were lower than those of pentacyclic triterpenes. No attempt was made to identify these low-M_r terpenes.

Table 7. Fatty acid compositions (% total) of the triglycerides of three species of mangrove grown normally and under tidal water

Component	*A. officinalis*		*A. illicifolius*		*B. gymnorhiza*	
	S*	N*	S	N	S	N
12:0	—	4.0	—	7.3	—	8.6
14:0	3.0	2.7	8.0	3.9	10.8	9.8
14:1	—	3.4	—	5.6	—	2.7
16:0	25.5	24.6	13.6	12.6	18.8	17.3
16:1	6.9	7.7	2.0	3.9	1.6	5.3
17:0	2.4	1.2	0.9	1.9	0.6	4.6
18:0	7.6	7.5	5.3	5.3	10.7	9.4
18:1	28.2	23.2	19.9	16.9	28.6	20.0
18:2	7.1	9.7	15.1	21.6	11.4	12.6
18:3	16.9	11.3	31.4	15.7	13.6	4.7
20:0	0.8	1.0	0.9	1.2	0.6	0.9
22:0	0.2	0.1	0.2	0.6	0.5	0.8
22:1	0.5	0.2	0.1	0.2	0.4	0.2
23:0	0.4	0.7	0.5	0.8	0.8	1.0
24:0	0.6	1.0	0.7	0.9	0.6	0.8
25:0	0.3	0.9	0.6	0.8	0.5	0.6
26:0	0.1	0.8	0.8	0.8	0.5	0.7

*S, Submerged under tidal water; N, normal.

In our previous study [1], it was observed that plants grown in water stressed condition, i.e. submerged periodically under tidal water, accumulated higher proportions of hydrocarbons and wax esters in the leaves. Moreover, other parameters, like leaf area, thickness, fresh weight to dry weight quotient, loss in weight on drying and chlorophyll content of leaves, were all higher in periodically submerged plants [1].

Deposition of higher proportions of hydrocarbons and wax esters may be helpful for controlling the entry of excess water into the leaves while submerged under water. Moreover, from the observations in the present study, particularly the leaf surface components, it is observed that, submerged plants synthesize proportionaltely more shorter carbon chain hydrocarbons and *n*-alcohols, along with higher proportions of unsaturated fatty acids in the wax esters. This is probably necessary for the leaves to maintain proper flexibility under water at a relatively lower temperature, compared to that of the surrounding atmosphere. Higher proportions of unsaturated fatty acids were also present in the steryl esters and triglycerides of submerged plant leaves. Moreover, only low-M_r terpenes were syntesized in the leaves of submerged plants.

From the results of the present study, it is revealed that, in general, the carbon–carbon chain elongating activity is decreased, except in the fatty acids of steryl esters, whereas the desaturating activity is increased in the submerged leaves compared to the leaves grown normally.

EXPERIMENTAL

Samples. 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. Leaf samples were collected from slopes where tidal water could reach only to the root zone. Other leaf samples of the same species were collected from plants on banks which were totally submerged during high tides. Leaves were washed thoroughly with distilled H_2O before analysis.

Isolation of terpenoids. Leaves were cut into pieces, and lipids were extracted as in ref. [3]. A heavy white ppt appeared upon dilution of the pooled extracts with 10 vols of H_2O. The lower $CHCl_3$ layer was withdrawn. The ppt was washed with a small volume of $CHCl_3$, dissolved in MeOH–$CHCl_3$ (2:1), dried and weighed [2]. The $CHCl_3$ layer was dried and weighed to give the total lipid.

Fractionation of total lipid. Prep. TLC was done to separate various lipid classes, as described in ref. [4]. Hydrocarbon, wax ester and steryl ester bands were partially resolved. Using Et_2O–*n*-hexane (1:49) they were then further fractionated by prep. TLC. The various lipid components were recovered from the plates and weighed.

Colour reactions of sterols and terpenoids. The Liebermann–Burchard [5, 6] test produced a greenish colour with sterols and a blue or violet colour with terpenes. Differentiation of sterols and terpenes by colour reactions was done according to ref. [7].

Analysis of hydrocarbons. Hydrocarbons were analysed by GLC according to ref. [8].

Analysis of wax esters and steryl ester. Wax esters were analysed as in ref. [9]. Steryl esters were analysed as in ref. [10].

Fatty acid compositions of triglycerides. Fatty acids of triglycerides were obtained by alkaline hydrolysis and were analysed by GLC as their methyl esters, as in ref. [8].

Table 8. Sterol compositions (% total) of three species of mangrove leaves grown normally and under tidal water

Component	*A. officinalis*		*A. illicifolius*		*B. gymnorhiza*	
	S*	N*	S	N	S	N
Unidentified	2.6	—	0.7	·	1.9	—
Cholesterol	4.2	7.0	0.7	4.9	0.7	1.7
Campesterol	7.2	9.0	5.7	19.8	15.4	22.7
Stigmasterol	10.7	12.8	22.7	26.0	15.4	17.7
Stitosterol	63.1	51.7	41.1	36.1	62.9	53.7
28-Isofucosterol	6.7	—	28.9	—	2.0	—
Stigmast-7-en-3β-ol	5.5	19.3	0.9	13.2	1.7	4.2

*S, Submerged under tidal water; N, normal.

Analysis of sterols and terpenes. The sterols and triterpenoids were analysed as in ref. [2].

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