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LIPID COMPOSITION OF *PORTERESIA COARCTATA* FROM TWO DIFFERENT MANGROVE HABITATS IN INDIA

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Key Word Index—*Porteresia coarctata*; Gramineae; sea-grass; mangrove ecosystem; lipids; fatty acids; sterols, terpenoids; environmental stress.

Abstract—*Porteresia coarctata* collected, at Prentice and Chuksar Islands of the Sunderban mangrove ecosystem, was studied for its lipid composition. Hydrocarbon, wax ester, sterol esters, triacylglycerols, sterols and terpenoids, were all found in higher proportions in the Prentice Island sample. Palmitic acid was the major fatty acid in all fractions except for the steryl esters of *P. coarctata* collected from Prentice Island, where oleic and linoleic acids were the major components. Sterols found were, sitosterol, stigmasterol, campesterol and cholesterol, of which, sitosterol and stigmasterol were the major components. Terpenoids present were, β-amyrin, α-amyrin, lupeol, betulin, oleanolic acid and ursolic acid, of which, lupeol and oleanolic acid were the major components. Quantitative variations of the lipid constituents were found between the two samples studied.

INTRODUCTION

Sea-grasses play an important role mainly in temperate regions and also within mangroves by providing a unique environment. Sea-grasses trap terrestrially derived inputs and produce significant quantities of materials of plant and animal origin, which are known to support micro-organisms and phytoplankton [1]. They provide food as live biomass and fix a large proportion of organic carbon, much of which enters the nutrient cycle either by direct feeding or grazing of animals or via decomposition as detritus [2].

Sea-grasses are important primary producers in the marine environment and provide food, shelter, nursery grounds and physical support for many crustaceans, fish fauna [3] and epiphytic organisms [4]. Sea-grasses and associated organisms have been extensively studied [5–13] for various lipid components, which may have the potential to be used as 'chemical markers'. It is suggested that various lipid components of *Posidonia australis* and *Heterozostera tasmanica* could be used as potential markers for monitoring sea-grass contribution to both food webs and marine sediments [5]. Detailed analyses of specific lipids of *Zostera muelleri* have been performed [9], the fatty acids and sterols of some sea-grasses [13], the sterols and fatty acids of seven tropical sea-grasses of Australia [14] and the lipid components of the leaves and roots of the tropical sea-grass, *Thallasia hemprichii*, collected from North Queensland [15] have been reported and investigated.

Porteresia coarctata is the only sea-grass found in the Sunderbans mangrove ecosystem, where it grows abundantly at Prentice and Chuksar Islands. The morphology of this plant, however, was found to be entirely different at the two habitats. The leaves were long, broad and thick in the Prentice Island sample whereas material from Chuksar Island had stunted growth with short and narrow leaves. The two islands are characterised by different environmental conditions. Mud and fine silt deposition occurs at Prentice Island, which is situated in a deltaic estuarine location. On the other hand, Chuksar Island is situated in an off-shore location, where sandy to fine sand sedimentation is found. Plants at Chuksar Island are in a high salinity regime and subjected to partial inundation during high tides, whereas, at Prentice Island total inundation was observed, which was similar to a previous study on adaptation of plants under tidal water [16].

This communication deals with the chemical comparison of various lipids isolated from *P. coarctata* growing in the two habitats of the Sunderbans mangrove ecosystem.

RESULTS AND DISCUSSION

Percentages of total lipids and various components of total lipids have been presented in Table 1, together with terpenoids which are not a part of the total lipid. The contents of hydrocarbons, wax esters, sterol esters, triacylglycerols, free sterols and terpenoids were much higher in the sample from Prentice Island. On the other hand, the polar lipids in the Chuksar Island sample, were more than double the amount present in the Prentice Island sample. Polar lipids were the major fractions in both the samples.

Hydrocarbon compositions of the two samples (Table 2) indicate that even carbon chain components predominate over the odd carbon chains, which is very unusual. Normally odd carbon chain hydrocarbons formed by the decarboxylation of even carbon chain fatty acids occur in higher proportions [17]. The predominance of even carbon chain hydrocarbons might be due to the formation of an excess of odd carbon chain fatty acids from even carbon chain fatty acids by the α-oxidation of the latter [18] followed by decarboxylation of the odd carbon chain fatty acids, leading to the formation of an excess of even

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Table 1. Compositions (μg/g fr. wt of leaves) of total lipids and other lipid components of *P. coarctata* growing in two habitats

Lipid components	Prentice Island	Chuksar Island
Hydrocarbons	1344	847
Wax esters	162	101
Steryl esters	4227	3367
Triglycerides	1008	995
Sterols	2430	1310
Polar lipids*	4329	9380
Total lipid	13500	16000
Triterpenoids†	9462	4456

*Polar lipids includes glyco- and phospholipids.
†Terpenes not included in total lipids.

carbon chain hydrocarbons. Similar observations were made in samples of an Antarctic species where even carbon chain hydrocarbons predominated in 80% of the samples analysed [19]. Predominance of even carbon chain hydrocarbons over the odd carbon chains in the present study is similar to previous findings in this ecosystem [20] and, therefore, probably is typical of this environment.

The fatty acid composition of the total lipids, triacylglycerols and sterol esters (Table 3), indicates that the chain lengths of the fatty acids were between C_{12} and C_{22}. Among the saturated and unsaturated fatty acids palmitic acid (16:0) is the major component in all the samples except in the sterol esters of the Prentice Island sample, where, 18:1 and 18:2 were the major components. 14:0 is present in higher proportions in all the samples from Chuksar Island. Another major component among the saturated acids is 18:0, which is present in higher amounts in samples from Prentice Island except in the sterol ester fraction. Among the unsaturated acids, 18:1, 18:2 and 18:3 are major components. The proportion of unsaturated fatty acids was always higher in the samples of Prentice Island, The greatest being 75.3% in the steryl esters indicating in turn that the environment at Prentice Island is favourable for the biosynthesis of unsaturated compounds. In the present study, low levels of 18:3 were recorded in the sea-grass samples compared to the results of refs [5, 14] in the sea-grasses of Victoria and Queensland, respectively. The 18:3 content of plants in the present study, might be low becasue of the higher temperature of the environment. It has been demonstrated by various authors that, low temperature favours biosynthesis of fatty acids with higher degree of unsaturation in plants [21–23]. The amount of 18:3 in *P. coarctata* from Sunderbans is comparable to that of some of the other plant leaves growing in this ecosystem [20, 24–26]. Analysis of wax esters was not done because their proportions were very low in the sea-grass samples.

Sterol compositions of the steryl ester and free sterol fractions of the plants from the two habitats are shown in Table 4; sitosterol and stigmasterol were the major components, typical of most higher plants and also in mangrove plant leaves [27]. 28-Isofucosterol found in all the seven tropical sea-grasses [14], was not found in the present study. The stereochemistry of sterols identified in the present study was not determined. Some sterol fractions (Table 4) contained appreciable amounts of unidentified components, which were also of a steroidal nature.

The common constituent triterpenes were β-amyrin, α-amyrin, lupeol, oleanolic acid and ursolic acid (Table 5). An unidentified component was also present in both sea-grass samples. Lupeol and oleanolic acid were the major triterpene constituents in both the samples. Betulin was only present in the sample from Chuksar Island. Total triterpenoid contents were relatively high (Table 1) but similar to those of mangrove plants reported earlier [27] and, therefore, probably typical of this estuarine environment. However, the content of triterpenoids in the sample from Prentice Island was more than double that of the

Table 2. Hydrocarbon compositions (% total fraction) of *P. coarctata* growing in two habitats

Components	Prentice Island	Chuksar Island	Components	Prentice Island	Chuksar Island
16:0	—	1.2	26-*anteiso*	1.0	0.3
17:0	—	0.9	26:0	1.3	4.8
18:0	2.3	2.9	27-*anteiso*	0.4	—
19:0	0.9	0.9	27-*iso*	0.1	—
20-*iso*	—	0.1	27:0	2.3	1.9
20:0	1.2	2.0	28-*iso*	3.4	2.4
21-*iso*	0.1	0.2	28:0	7.0	7.3
21:0	1.2	1.5	29-*iso*	0.3	0.2
22-*anteiso*	0.1	0.1	29:0	3.5	2.5
22-*iso*	0.1	0.3	30-*iso*	4.0	2.5
22:0	1.1	1.4	30:0	10.2	13.2
23-*anteiso*	0.1	0.1	31-*iso*	0.2	—
23-*iso*	0.1	0.3	31:0	2.3	1.8
23:0	1.7	1.3	32:0	11.5	13.4
24-*anteiso*	0.2	0.1	33:0	2.8	2.3
24-*iso*	0.5	0.1	34:0	18.5	14.2
24:0	8.0	6.4	35:0	1.4	1.4
25-*anteiso*	0.2	0.1	36:0	7.7	8.6
25-*iso*	0.1	Tr.	37:0	0.9	0.9
25:0	2.6	1.6	38:0	0.7	0.8

Table 3. Fatty acid compositions (% total fraction) of the total lipids, triacylglycerols and steryl esters of *P. coarctata* growing in two habitats

Components	Total lipids		Triacylcerols		Steryl esters	
	Prentice Island	Chuksar Island	Prentice Island	Chuksar Island	Prentice Island	Chuksar Island
12:0	1.6	4.0	2.9	2.1	1.2	2.3
14:0	6.0	15.2	5.1	17.4	1.1	8.6
15:0	1.2	7.0	0.8	1.0	1.7	0.5
15:1	0.6	0.8	0.6	—	—	—
16:0	36.5	35.3	26.6	39.6	12.1	24.9
16:1ω7	2.5	2.3	0.8	7.8	4.2	4.6
17:0	2.0	1.0	—	1.5	1.1	4.4
17:1	1.2	0.5	—	—	—	—
18:0	8.4	6.3	26.6	10.0	1.8	16.5
18:1ω9	14.0	13.4	15.6	13.0	33.5	16.0
18:2ω6	11.3	10.0	4.0	5.0	30.6	1.7
18:3ω3	11.6	2.2	17.0	2.6	7.0	10.0
20:0	0.5	0.5	—	—	—	6.5
22:0	1.5	0.7	—	—	2.3	1.8
22:1ω11	1.0	0.8	—	—	3.4	2.2

Chuksar Island sample. This may be due to the fact that the grasses at the former site are totally submerged for *ca* 4–5 hr a day during high tides and thus are in a tidal water stress [16] and biosynthesize higher proportions of triterpenoids [20]. The occurrence of triterpenoids in sea-grass has not been reported previously. The high triterpene contents in plants of this ecosystem warrant further study.

The sea-grasses grow near the banks in both the islands. The average tidal height at Prentice Island is 5.0 m, whereas, that in Chuksar Island is only 2.5 m. The grasses are totally submerged at Prentice Island during high tide and, therefore, receive a reduced photon flux density in the turbid estuarine water at a low salt concentration. The periodic submergence subjects the plants to stress to which it adapts itself by making certain biochemical changes [16, 20].

The grasses at Prentice Island were found to biosynthesize higher proportions of lipid components and unsaturated fatty acids, for adaptation under tidal water stress, similar to the observations made in a previous study [20].

On Chuksar Island, plants experience less stress, because of partial submergence under tidal water at a high salt concentration due to the off-shore location of the island. It was pointed out by Strogonov [28] that high salt concentrations lead to accumulation of toxic products and as a result, the plants become small and retarded in development. This is observed at Chuksar Island.

Table 4. Sterol compositions (% total fraction) of steryl esters and free sterols of *P. coarctata* growing in two habitats

Components	Steryl ester		Free sterol	
	Prentice Island	Chuksar Island	Prentice Island	Chuksar Island
Unidentified	12.6	6.0	2.8	4.2
Cholesterol	15.2	8.5	8.3	5.2
Campesterol	5.2	7.5	11.7	14.0
Stigmasterol	23.0	30.0	20.5	25.3
Sitosterol	44.0	48.0	54.1	40.6
Unidentified	—	—	2.6	10.7

Table 5. Pentacyclic triterpenoid composition (% total fraction) of *P. coarctata* growing in two habitats

Components	Prentice Island	Chuksar Island
Unidentified	12.5	7.0
β-Amyrin	9.5	12.0
α-Amyrin	7.8	10.5
Lupeol	40.0	28.0
Betulin	—	11.0
Oleanolic acid	18.5	27.0
Ursolic acid	11.7	4.5

EXPERIMENTAL

Plant material. Samples of *P. coarctata* Roxb. were collected from Prentice Island, between latitudes 21.43 to 21.46° N and longitudes 88.18 to 88.19°E and Chuksar Island between latitudes 21.32 to 21.34°N and longitudes 88.0 to 88.2°E of the Sunderbans mangrove forest, West Bengal, India. Leaves were washed thoroughly with dist. H_2O and cut into pieces before lipid extraction.

Extraction of lipids and isolation of triterpenoids. Leaves were homogenized in MeOH–$CHCl_3$ (2:1), centrifuged and the residue rehomogenized with MeOH–$CHCl_3$–H_2O (2:1:0.8) [29]. After centrifuging, the residue was finally homogenized with the first solvent system and centrifuged. The supernatants were pooled and dil. with 10 vol. of H_2O, when a heavy white ppt. appeared [27]. The lower $CHCl_3$ layer was withdrawn and the white ppt. washed × 2 with small vols of $CHCl_3$. These washings were continued with the original $CHCl_3$ layer which contained the lipids. The white ppt. was dissolved in MeOH–$CHCl_3$ (2:1), dried and weighed to give the pentacyclic triterpenoids [27].

Fractionation of total lipid. Prep. TLC was used to sep. various lipid classes using petrol (40–60°)–Et_2O–HOAc (80:20:1.5) [30].

Hydrocarbon, wax ester and steryl ester bands were partially resolved further fractionated by prep. TLC using Et_2O–*n*-hexane (1:49). The various lipid components were recovered from the adsorbent and weighed.

Hydrocarbons were analysed directly by GC on a 3% OV-17 column; temp prog. from 215 to 265° at 4° min [31]. Identifications were made using appropriate stds both external and internal.

Steryl esters. Lipolysed on TLC plates using porcine pancreatic lipase; sterols and fatty acids were separated on the same TLC plate and were recovered as in ref. [25].

Fatty acids. Derived from steryl esters and triacylglycerols were analysed as their Me esters on a 10% DEGS column, (1.8 m × 3 mm) operated at 180°. Identifications were made using authentic stds both external and internal [31]. Unsaturated homologues were confirmed by catalytic hydrogenation [32] and rechromatography under identical conditions.

Sterol acetates were prepared by refluxing the sterols with Ac_2O for 30 min [33]. The derivatives were analysed on a 3% SE-30 column at 285° with a N_2 flow rate of 60 ml/min. Identifications were made using RR_ts (cholesteryl acetate) and comparing them with those reported by Patterson [33]. Co-chromatography with acetates of authentic sterols was also performed for confirmation. Further Identification of sterols was carried out by GC of the corresponding TMSi ethers [34] on a 3% OV-17 column at 255° with a N_2 flow rate of 60 ml/min. Retention indices (RI) were determined [35] and comparisons made with those reported in ref. [36].

Triterpenoids. Analysed on a 3% OV-17 column at 290 (acetate derivatives) and 284° (TMSi ether derivatives) with a N_2 flow rate of 60 ml/min. Identifications were made from comparisons of the RR_ts and RI of the compounds with those reported in ref. [27].

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