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FATTY ACIDS, STEROLS AND HYDROCARBONS IN THE LEAVES FROM ELEVEN SPECIES OF MANGROVE*

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Key Word Index—Mangroves, *Avicennia*, *Xylocarpus*, *Rhizophora*, *Ceriops*, *Bruguiera*, *Acanthus*, *Sonneratia*, fatty acids, sterols, hydrocarbons, chemotaxonomy

Abstract—The fatty acid, sterol and hydrocarbon compositions of the fresh leaves from eleven species of mangroves, cultivated in a shadehouse, are reported The fatty acid and sterol analyses, whilst generally typical of higher plants, showed several chemotaxonomically significant differences between the species The epicuticular wax hydrocarbons and fatty acids were low in abundance compared to previous reports of mangrove leaf lipids, which may reflect the importance of environmental influences on this group of compounds Cluster analysis of selected subsets of the data showed clear chemotaxonomic divisions amongst the mangroves The results grouped the mangroves into genera, except for the *Rhizophora* and *Ceriops tagal* which were not separated, and grouped the family Rhizophoraceae distinct from all other species except *Xylocarpus granatum* *Avicennia marina* var *resinifera* was able to be distinguished from *Avicennia marina* by cluster analysis, supporting its assignment as a distinct variety The results show that the lipids of mangroves are chemotaxonomically significant

INTRODUCTION

Mangroves are widespread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coastlines They have a high rate of primary productivity and provide a substantial input of detrital material into the surrounding ecosystem Litter fall rate experiments by Bunt *et al* [1, 2] have measured this input to be *ca* 8 tonnes (dry wt)/ha per year in north Queensland, and measurements from other regions of the world have shown similar rates of litter production [3] This mangrove detrital material provides an important source of both particulate and soluble organics into the local environment Snedaker [4], in reviewing the ecological importance of the mangrove ecosystem, discussed the value of this organic material in forming the basis of a complex food web

Information about the nature of organics arising from mangroves was provided by Wannigama *et al* [5], who reported detailed analyses for a number of lipid classes present in the leaves and pneumatophores of the mangrove *Avicennia marina* growing at Port Franklin Johns and Onder [6] had previously shown that this plant was the main source of dicarboxylic acids in the surrounding sediments In a study by Sassen [7, 8], selected tissues from several mangrove species were analysed to evaluate the mangrove contribution of fatty acids into associated sediments Lignins, characterized as phenolic oxidation products, have also been studied in a mangrove plant It was reported by Hedges *et al* [9, 10] that the lignin oxidation product, *p*-coumaric acid, was present at a higher concentration in *Avicennia germinans* than in any of the other plants examined Lignins, because of their relative stability, have been used as an indicator of land-derived organic matter in recent marine sediments [9, 11]

As part of our study of the ecology of coastal regions in north Queensland [12], we required detailed analyses of the lipids present in mangrove leaves so that we could accurately assess the importance of mangrove contributions to the ecosystems Current studies indicate that there are between 30 and 40 different mangrove species growing along the Queensland coast [13, 14] It is possible that the chemistry of the plants may differ and so it was important to consider several species Such a study would also be useful for chemotaxonomy There are difficulties and debate about the taxonomy of mangroves growing in this region as, for instance, a number of the species appear in morphologically different forms [14] Lipid analytical studies may provide some insight into these problems In this paper we report selected lipid analyses of the fresh leaves of 11 species of mangrove cultivated in a shadehouse Ten of these species can be found in the wild state along the north Queensland coast, whilst one, *Avicennia marina* var *resinifera*, occurs in New Zealand

RESULTS AND DISCUSSION

Fatty acids

Detailed analyses of the fatty acids present in the mangrove leaves are reported in Table 1 The acids found ranged from C_{12} to C_{24}, with the major acids present (> 90% of the total acids observed) being 16 0, 18 0, 18 1 (9), 18 2 (9, 12) and 18 3 (9, 12, 15) as has previously been reported for other mangrove species [5, 7, 8] The relative concentration of 18 2 (9, 12) was lower in the two *Bruguiera* species than in the other species, whilst *Acanthus ilicifolius* and *Sonneratia caseolaris* yielded,

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Table 1 Fatty acid composition of mangrove leaves

Homologue*	ECL†	ECL‡	Percentage of total fatty acids										
			Am §	*Ar*	*Rs*	*Ra*	*Rm*	*Ct*	*Bs*	*Bg*	*Xg*	*Ai*	*Sc*
12 0	12 00	12 00	tr	tr	0 2	0 2	0 2	0 2	0 3	0 6	0 4	0 1	tr
Unidentified	13 47	—	2 0	—	—	—	—	—	—	—	—	—	—
14 0	14 00	14 00	0 8	0 4	1 0	1 1	1 3	0 9	0 7	2 9	0 8	0 9	0 5
Unidentified	—	14 50	0 4	1 6	—	—	—	—	—	—	—	—	—
15 0	15 00	15 00	tr	tr	0 1	tr	0 1	0 2	tr	0 1	0 1	0 2	0 1
Unidentified	15 44	16 77	0 6	—	—	—	—	—	—	—	—	—	—
16 1(7)	15 71	16 18	0 1	0 1	0 2	0 1	0 2	0 1	0 1	0 1	0 2	tr	0 1
16 1(9)	15 75	16 24	0 7	1 0	0 6	0 4	0 4	0 3	0 3	0 1	0 3	0 3	0 8
16 1(3*t*)	15 92	16 50	1 1	1 1	0 9	0 4	1 1	0 7	0 8	1 1	0 6	1 2	1 4
16 0	16 00	16 00	23 1	22 2	28 4	23 9	28 5	29 0	25 2	26 0	25 6	20 2	28 2
17 1(9)	16 78	17 20	0 3	0 1	0 2	0 2	0 2	0 2	tr	0 1	0 2	0 2	0 2
17 0	17 00	17 00	0 6	0 3	0 6	0 5	0 5	0 3	0 2	0 4	0 6	0 4	0 3
18 3(9)	17 66	19 23	38 5	32 9	41 8	43 3	34 9	34 6	46 4	43 9	45 7	43 2	41 8
18 2(9)	17 61	18 61	17 6	21 5	12 6	16 6	20 5	15 1	11 1	11 2	13 7	23 6	18 3
18 1(9)	17 71	18 19	8 8	10 6	8 6	8 8	7 0	12 0	9 2	8 1	8 3	2 6	3 6
18 1(11)	17 76	18 24	0 5	0 4	0 4	0 5	0 6	0 5	0 2	0 4	0 2	0 7	0 5
18 0	18 00	18 00	2 8	3 8	3 2	1 9	2 1	2 3	3 3	3 7	1 8	3 8	2 4
19 0	19 00	19 00	0 2	0 1	tr	tr	tr	tr	tr	0 1	tr	0 1	tr
20 3(11)	19 66	21 26	tr	0 1	0 2	tr	0 1	0 4	0 2	tr	0 1	tr	tr
20 2(11)	19 61	20 63	tr	0 1	tr	tr	tr	0 4	tr	—	0 2	—	tr
20 1(11)	19 70	20 17	tr	0 2	—	—	—	0 4	tr	—	tr	—	tr
20 0	20 00	20 00	1 0	1 0	0 2	0 1	0 2	0 2	0 2	0 2	0 2	0 4	0 5
21 0	21 00	21 00	0 1	0 1	—	—	—	—	—	—	—	tr	tr
22 1(13)	21 71	22 19	—	—	—	—	—	tr	—	—	—	—	—
22 0	22 00	22 00	0 2	0 2	tr	tr	tr	0 1	tr	tr	tr	0 1	0 2
24 0	24 00	24 00	—	—	—	—	—	—	tr	—	—	—	—
Minor unidentified (total)‖			0 2	2 2	0 8	1 7	2 0	2 1	1 5	0 7	0 8	1 7	0 7
Total absolute abundance μg/g (fr wt)¶			4900	5100	6400	13 100	5500	2900	7600	3400	3500	3600	2000

tr = trace, < 0 1%, — = = < 0 05%

*Double-bond positions, shown in parentheses, are numbered from the acid group All subsequent double bonds are methylene interrupted 16 1 (3*t*) = *trans*-double bond

†Equivalent chain length, SP2100 column

‡Equivalent chain length, Superox 0 1 column

§*Am* = *A marina*, *Ar* = *A marina* var *resinifera*, *Rs* = *R stylosa*, *Ra* = *R apiculata*, *Rm* = *R mucronata*, *Ct* = *C tagal*, *Bs* = *B sexangula*, *Bg* = *B gymnorrhiza*, *Xg* = *X granatum*, *Ai* = *A ilicifolius*, *Sc* = *S caseolaris*

‖ Each component < 0 15% relative concentration

¶The mean moisture content for leaves taken from the mangrove plants were *Am*, 65, *Ar*, 66, *Rs*, 66, *Ra*, 72, *Rm* not determined, *Ct*, 67, *Bs*, 58, *Bg*, 79, *Ai*, 78, *Sc*, 72, *Xg*, not determined

comparatively, very low relative concentrations of 18 1 (9) In *Ceriops tagal* the three C_{20} unsaturated acids were appreciably more abundant than in the other species, where they were generally present in only trace concentrations In both *Avicennia* species 20 0 was found in higher relative concentration (1%) than in any other species (< 0 5%) The *Avicennia* species were also characterized by three, as yet unidentified acids One of these was found in both species, whilst the other two were found only in *Avicennia marina* The total absolute abundance of fatty acids was considerably higher in *Rhizophora apiculata* (46 800 μg/g dry wt) than in any of the other species, whilst in *Sonneratia caseolaris* (7100 μg/g dry wt) and *Ceriops tagal* (8800 μg/g dry wt) the abundances were much lower The remaining species had total abundances (14 000–18 000 μg/g dry wt) of fatty acids which were comparable to those previously reported for mangrove leaves [5, 7, 8]

The fatty acid distribution in the two *Avicennia* species was very similar to that previously reported for *Avicennia marina* growing in Port Franklin [5], except that there were only trace amounts of long-chain acids (C_{23}–C_{32}) present These long-chain acids had also been observed in the leaves of several other mangrove species [7, 8] and were reported as being derived from the epicuticular wax In our study, however, long-chain fatty acids were only very minor constituents in the leaves of any of the plants examined

Sterols

Sitosterol was the major sterol component in all of the

species analysed (see Table 2), as could be expected for higher plants [15] The relative concentrations of the remaining sterols varied markedly from species to species *Rhizophora mucronata* contained a noticeably higher concentration of 28-isofucosterol than the other species, whilst the concentration of cholesterol was highest in *Acanthus ilicifolius* and *Sonneratia caseolaris* In the *Avicennia* species, the relative concentration of stigmasterol was higher than in most other species, whereas that of campesterol was lower Hence, the *Avicennia* species were distinguished from the other species by a high stigmasterol campesterol ratio Unidentified components constituted a significant proportion of the sterol fraction in the mangrove leaves, particularly in *Xylocarpus granatum* Projected future GC/MS analyses will enable identification of these compounds

The total abundance of sterols in the mangrove leaves ranged from *ca* 500 to 2500 μg/g dry wt For *Avicennia marina* the abundance (*ca* 900 μg/g dry wt) was only about half of that previously reported for *Avicennia marina* [5], although in *Avicennia marina* var *resinifera* the abundance was similar to that of the earlier report The relative distribution of the sterols in the two *Avicennia* species was very similar to that of the Port Franklin study [5] The observed abundance differences between the *Avicennia* samples possibly relate to environmental effects

Hydrocarbons

The dominant component of the hydrocarbon fraction was squalene (> 50% in 8 of 11 species), whilst the remaining major hydrocarbons had ECL values between 29 00 and 31 00 Abundances of selected hydrocarbons in the leaves are reported in Table 3 Most of the leaf hydrocarbons are believed to be derived from the epicuticular wax [5], the major exception being squalene

Table 2 Sterol composition of the mangrove leaves

	Percentage of total sterols										
Sterol	*Am**	*Ar*	*Rs*	*Ra*	*Rm*	*Ct*	*Bs*	*Bg*	*Xg*	*Ai*	*Sc*
Cholesterol	1 4	0 5	1 3	0 5	0 5	1 0	1 0	0 8	1 6	7 1	3 7
Campesterol	0 9	1 4	8 4	5 3	6 8	7 6	15 8	12 9	15 2	15 5	2 7
Stigmasterol	22 2	28 6	7 6	6 4	4 4	7 3	19 6	16 8	6 6	23 4	11 4
Sitosterol	66 7	61 8	72 1	80 7	76 9	75 8	60 8	66 0	54 8	47 8	78 9
28-Isofucosterol	2 3	3 0	3 8	4 3	9 0	3 7	1 8	2 0	2 1	4 7	1 3
Total unidentified	6 5	4 7	6 8	2 8	2 4	4 6	1 0	1 5	19 7	1 5	2 0
Total absolute abundance μg/g (fr wt)	320	570	410	690	320	270	440	310	500	350	150

*For species code see Table 1, footnote §

Table 3 Hydrocarbon composition of mangrove leaves

	Absolute composition μg/g (fr wt)										
Homologue	*Am**	*Ar*	*Rs*	*Ra*	*Rm*	*Ct*	*Bs*	*Bg*	*Xg*	*Ai*	*Sc*
25 0	0 4	0 6	0 6	2 1	—	0 3	tr	0 6	0 4	0 3	1 5
26 0	tr	—	—	2 3	—	—	—	0 8	—	tr	0 4
27 0	tr	0 2	—	5 2	1 3	—	tr	2 1	—	tr	0 3
28 0	nad	nad	nad	nad	nad	nad	nad	nad	nad	nad	nad
29 0	1 0	3 2	2 4	24 7	15 5	tr	2 4	1 5	2 8	2 3	0 7
30 0	1 5	0 4	0 1	—	3 3	—	1 2	0 4	1 4	1 5	1 0
31 0	12 5	42 2	1 2	141	14 6	0 4	2 1	4 2	3 6	13 1	2 4
32 0	2 6	7 2	—	30 4	1 3	—	—	1 0	—	3 0	—
33 0	31 0	81 4	—	53 0	3 4	—	—	2 8	—	11 2	—
34 0	1 4	2 1	—	1 4	—	—	—	—	—	0 4	—
35 0	4 4	3 0	—	—	—	—	0 9	0 5	—	1 0	—
Squalene	368	155	914	2243	678	218	213	1688	9 1	18 6	70
Others	89	94	47	123	183	26	48	78	17	154	62
Total	512	389	966	2626	901	245	268	1780	34	206	138
Total epicuticular wax hydrocarbons†	144	234	52	383	223	27	55	92	25	187	68

tr = trace, < 0 2 μg/g (fresh wt), nad = not accurately determined

*For species code see Table 1, footnote §

†Total epicuticular wax hydrocarbons = total hydrocarbons − squalene (refer to text)

The total abundance of leaf-wax hydrocarbons, therefore, was estimated as the total hydrocarbon abundance minus squalene, and ranged from *ca* 80 to 1400 μg/g dry wt The values for *Avicennia marina* (411 μg/g dry wt) and *Avicennia marina* var *resinifera* (688 μg/g dry wt) were much lower than equivalent results for *Avicennia marina* from Port Franklin [5], and abundances in other species were lower than anticipated on the basis of this previous report We have already noted that the longer-chain epicuticular wax acids were very minor in our analyses This apparent paucity of epicuticular wax lipids may be the result of selection for low-wax species in the tropical climate or may be a result of the cultivation conditions

Cluster analysis of analytical data

Cluster analysis was performed on selected subsets of the analytical data to test whether lipid analyses could readily distinguish the mangrove genera and possibly species Data subsets were evaluated on their ability to distinguish genera and species, with the data for *Avicennia marina* reported by Wannigama *et al* [5] being included as an additional test The best data set was found to consist of the percentage abundances of the acids, 16 0, 18 0, 20 0, 18 1 (9) and 18 2 (9, 12), together with the percentage abundances of all the identified sterols Hydrocarbon analyses were not included since our results indicated that this lipid class may be strongly influenced by environmental effects All data were subjected to a logarithmic transform and then standardized to *z*-scores prior to cluster analysis Euclidean distance was chosen as the criterion for cluster combination Figure 1 depicts the resultant tree diagram of the clusters The vertical axis of Fig 1 is a measure of the Euclidean distance between amalgamated clusters

Examination of Fig 1 shows that the two samples of *Avicennia marina* were separated by a distance of approximately 1 4σ whilst the two *Bruguiera* species differed by only 0 9σ It is thus evident that geographical variations in the sterol and fatty acid compositions of the mangroves may obscure species differences, however, this example is an extreme case because *Avicennia marina* at Port Franklin is a small bush (*ca* 1 m tall) growing in stress conditions, whereas in the tropics *Avicennia marina* commonly grows to a tree, 3–5 m tall Most of the other mangrove species occur over geographically more limited ranges We are currently examining further samples of mangroves to test intra-species variability and geographical effects The variety, *Avicennia marina* var *resinifera*, is native to a latitude intermediate between the other two *Avicennia marina* samples, and thus its clear separation from these samples supports its classification as a distinct variety

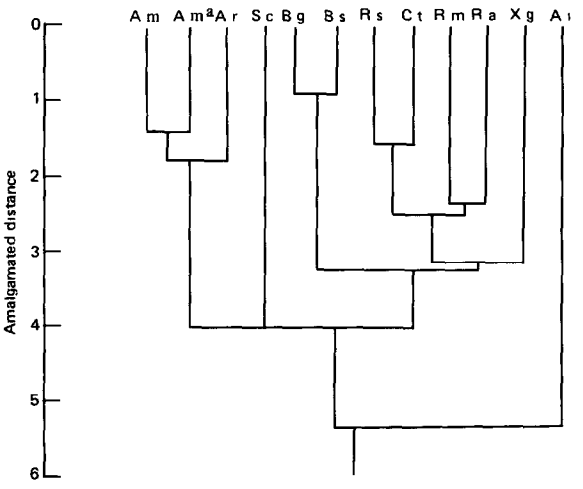

Fig 1 Tree diagram from cluster analysis of lipid data, based on fatty acids [16 0, 18 0, 20 0, 18 1 (9), 18 2 (9,12)] and sterols For explanation see text a, Data of Wannigama *et al* [5]

At an amalgamated distance of 3 0σ, all genera other than *Rhizophora* and *Ceriops* are well separated Triterpenoid analyses clearly distinguish these genera and will be reported in a subsequent publication At a distance of 3 5σ, the Rhizophoraceae (*Rhizophora*, *Bruguiera* and *Ceriops* species) plus *Xylocarpus* join into a distinct group, supporting the proposition that lipid analyses may provide a clear chemotaxonomic division of the various mangroves into genera and families Further studies of other chemical components present in the mangroves are in progress These initial results support the view that the lipids of mangroves are chemotaxonomically significant

EXPERIMENTAL

Fresh leaves, for lipid analyses, were taken from mangrove plants cultivated from seedlings by Dr B Clough at the Australian Institute of Marine Science, Townsville, Queensland The species examined were *Avicennia marina*, *Avicennia marina* var *resinifera*, *Xylocarpus granatum*, *Rhizophora stylosa*, *Rhizophora apiculata*, *Rhizophora mucronata*, *Ceriops tagal* var *australis*, *Bruguiera sexangula*, *Bruguiera gymnorrhiza*, *Acanthus ilicifolius* and *Sonneratia caseolaris* Moisture content was determined on separate leaf samples (mean of 3 samples) The leaves were cut into small pieces and ground in an agate mortar and pestle under $CHCl_3$–MeOH (1 1) The ground leaves were extracted twice with $CHCl_3$–MeOH (1 1) to give a total soluble extract, which was concd to a small vol under N_2 An aliquot of the solvent extract was saponified and partitioned [16] to give the neutral lipids and the acidic lipids Sterols and hydrocarbons were isolated from the neutral fraction by normal-phase HPLC using a CN-bonded column [250 mm × 4 6 mm i d , Spherisorb CN (5 μm)], under isocratic conditions with *iso*-octane–PrOH (99 1, 1 0 ml/min) as eluant UV absorbance was monitored at 210 nm

The acidic lipids were methylated with H_2SO_4–MeOH and analysed by GC using a fused silica WCOT SP2100 column (50 m × 0 2 mm i d) as previously described [17], and a fused silica WCOT Superox 0 1 column (50 m × 0 2 mm i d) The columns were programmed from 150° to 270° at 2°/min The sterols were treated with MeI–18-crown-6 ether–K tert-butoxide (in DMSO, 60°, 15 min) Two extractions with *iso*-octane–$CHCl_3$ (4 1) yielded the steryl methyl ethers, which were analysed by GC using the SP2100 column with programming from 150° to 270° at 10°/min, followed by a 30 min isothermal period at 270° during which the methyl ethers eluted The hydrocarbons were also analysed using the SP2100 column, with programming from 150° to 270° at 2°/min H_2 was used as carrier gas (linear flow 35 cm/sec) Eluting peaks were detected by FID, with injector and detector temps set at 280° The methyl esters were identified as previously described [17] Steryl methyl ethers were identified by comparison of chromatographic R_ts with those of authentic standards, and the data of ref [5] Authentic standards of sterols were courtesy of the Medical Research Council (U K)

Hydrocarbon identifications were based upon comparisons of their ECL values with standards, where available

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