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A COMPARISON OF LIPID COMPONENTS OF THE FRESH AND DEAD LEAVES AND PNEUMATOPHORES OF THE MANGROVE *AVICENNIA MARINA*

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Key Word Index—*Avicennia marina*; Avicenniaceae; mangrove; hydrocarbons; alcohols; sterols; triterpene alcohols; monobasic acids; α,ω-dibasic acids; ω-hydroxy acids.

Abstract—The component hydrocarbons, sterols, alcohols, monobasic, α,ω-dibasic and ω-hydroxy acids of the fresh and decayed leaves and the pneumatophores of the mangrove *Avicennia marina* are reported in detail. From the quantitative comparisons which can be drawn, relative changes in the lipid classes occurring during leaf decay can be highlighted. These base-line data are important to our understanding of inputs to marine intertidal sediments. During leaf decay the only significant changes were a reduction in the total absolute concentrations of monobasic acids due largely to a decrease in concentration of the C_{18} polyunsaturated fatty acids, and an enhancement of the concentrations of the long-chain monobasic acids, ω-hydroxy acids and α,ω-dibasic acids. This resistance to degradation shown by the cutin derived acids (α,ω-dibasic, ω-hydroxy and long-chain monobasic acids) relative to the cellular and wax derived lipids may allow these cutin components to be used as quantitative markers of *A. marina* in mangrove associated sediments.

INTRODUCTION

In the course of the studies of lipid input and diagenesis in mangrove associated sediments we have previously reported an analysis of the diacids of the mangrove *Avicennia marina* [1] and proposed direct input of the diacids to the sediments. A study by Sassen on mangrove peats [2,3], involving analysis of monobasic acids only, suggested a major input of mangrove lipids to a sediment, and presented evidence (leaf litter analysis) of selective degradation of short-chain fatty acids. The mangroves *Rhizophora mangle, Laguncularia racemosa, Avicennia germinans* and *A. nitida* were analysed. Similar degradative studies have been reported on the sea grass *Spartina alterniflora* [4], and on leaves in fresh water streams [5,6].

In this paper a more detailed lipid analysis of the living and dead leaves and the pneumatophores of *Avicennia marina* collected from Port Franklin, Victoria is reported. Lipid classes analysed include hydrocarbons, sterols, fatty alcohols, monobasic acids, α,ω-dibasic acids and ω-hydroxy acids. The fatty acids and alcohols were further subdivided into bound and solvent-extractable fractions, as prior work [7] has suggested the interpretative usefulness of this procedure. The monobasic acid data reported includes the molecular structures of the component fatty acids, since such data are essential for both biochemical and geochemical interpretation of the components present and of the changes observed during mangrove biodegradation and early diagenesis in recent marine sediments [7]. Such knowledge is pertinent to models of lignite coal deposition.

RESULTS AND DISCUSSION

The component hydrocarbons of the fresh and dead leaves and the pneumatophores of *Avicennia marina* are presented in Table 1. The hydrocarbon distributions in the leaf wax and in the leaves after wax removal are also presented. The *n*-alkane distribution of the fresh leaves is similar to those reported for the waxes of most higher plants [8–10]. Features such as the abundance of branched alkanes and the presence of long-chain alkenes do however make the hydrocarbon profile of *A. marina* distinctive. The paucity of previous alkane reports which include data on these components may be the result of the insufficient resolution of the packed GC columns previously employed for such analyses. However, one example is the noting of branched alkanes in the epicuticular wax of *Andropogon hallii* [11]. The hydrocarbon distribution in the dead leaves is similar to that found in the fresh leaves even to the presence of branched alkanes and traces of long-chain length alkenes. The leaf wax contrasts with the whole leaf analysis only in the absence of squalene.

The component hydrocarbons in the pneumatophores are characterized by a predominance of shorter chain *n*-alkanes. Long-chain *n*-alkanes, *n*-alkenes and branched alkanes were not detected in contrast to the leaves. Squalene was present, but at a reduced relative level compared to the leaf samples. These differences can be rationalized in terms of the different structure and function of these two plant organs.

The sterol composition of the fresh and dead leaves of *A. marina* (Table 2) are extremely similar. Sitosterol (the

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Table 1. Hydrocarbon composition of *Avicennia marina*

Homologue	Percentage composition of total hydrocarbons				
	Fresh leaves (TSE)	Fresh leaves (wax)	Fresh leaves (TSE − wax)	Dead leaves	Pneumatophores
n-C_{17}	—	—	—	—	0.22
n-C_{18}	—	—	—	—	0.19
n-C_{19}	—	—	—	—	0.72
n-C_{20}	—	—	—	—	0.30
n-C_{21}	tr	—	tr	0.14	3.5
n-C_{22}	tr	—	tr	tr	3.0
n-C_{23}	0.19	tr	0.14	1.2	29.6
n-C_{24}	0.10	tr	tr	0.20	4.8
n-C_{25}	0.52	0.39	0.29	2.2	35.0
n-C_{26}	tr	0.14	0.10	0.20	2.9
iso-C_{27}	0.17	tr	tr	tr	—
n-C_{27}	0.84	0.71	0.50	1.8	11.5
anteiso-C_{28}	0.19	0.10	tr	0.14	—
n-C_{28}	0.36	0.33	0.14	0.43	0.11
iso-C_{29}	0.29	0.43	0.34	0.13	—
n-C_{29}	4.0	4.0	2.9	7.0	0.46
anteiso-C_{30}	4.4	9.5	5.5	7.7	—
n-C_{30}	2.3	1.3	0.80	2.6	—
iso-C_{31}	2.2	1.4	1.2	1.5	—
n-C_{31}	27.5	36.9	26.6	25.3	—
anteiso-C_{32}	2.7	3.9	3.4	tr	—
n-C_{32}	3.7	3.5	2.9	2.9	—
iso-C_{33}	0.10	0.11	nad	tr	—
n-C_{33}	17.1	35.8	23.3	12.6	—
anteiso-C_{34}	0.16	0.15	nad	0.20	—
n-C_{34}	0.49	0.57	nad	0.79	—
n-C_{35}	0.56	0.61	nad	1.1	—
n-$C_{28:1}$	0.16	nad	nad	0.32	—
n-$C_{30:1}$	0.76	nad	nad	1.36	—
n-$C_{32:1}$	0.28	nad	nad	0.60	—
Squalene	30.8	—	31.7	32.1	6.8
Others	tr	tr	tr	4.0	0.9
Total absolute concn μg/g (dry wt.)	3070	—	—	3200	—

tr = Trace, <0.1%.
nad = Not accurately determined.
TSE = Total solvent extract.

configuration at C_{24} is assumed to be the same as that observed in other higher plant analyses) and stigmasterol are the two major sterol components, with campesterol and 28-isofucosterol also found to be present. This observed sterol distribution is similar to that found in other higher plants [12]; 28-iso-fucosterol has been previously reported in Liliaceae [13]. Virtually no sterols were detected in the epicuticular wax faction of the fresh leaves. The similar ratio of Δ5,22E/Δ5 sterols and absolute amounts of sterols found in the fresh compared with the dead leaves suggest that aerobic oxidation of sterols is a minor process since one would expect the more unsaturated sterols to degrade more rapidly under aerobic conditions.

The sterol distribution in the pneumatophores is dominated by sitosterol (87%) with smaller amounts of stigmasterol (11%) and campesterol detected. This profile

Table 2. Sterol composition of the leaves of *Avicennia marina*

Sterol	Percentage composition of total sterols	
	Fresh leaves	Dead leaves
Cholesterol	1.6	1.1
Campesterol	0.7	0.8
Stigmasterol	22.7	26.6
Sitosterol	70.2	66.2
28-Isofucosterol	2.3	2.0
Unidentified	2.3	3.1
Total absolute concn μg/g (dry wt.)	1900	1640

Table 3. Fatty alcohol composition of *Avicennia marina*

Homologue	Percentage composition of total alcohols						
	Fresh leaves				Dead leaves		
	TSE	Wax	(TSE – wax)	Bound	TSE	Bound	Pneumatophores
12:0	0.25	—	—	0.25	0.32	—	—
14:0	—	—	—	tr	0.60	—	—
15:0	0.17	—	—	tr	tr	tr	—
16:0	0.13	—	—	0.13	0.27	0.30	—
18:0	1.88	—	—	0.67	0.40	0.58	—
18:1	—	—	—	tr	—	0.13	—
20:0	0.88	0.66	0.7	3.41	1.80	3.96	7.9
21:0	tr	tr	tr	0.42	tr	1.40	7.3
22:0	0.84	2.0	1.3	5.45	1.77	4.66	12.7
23:0	0.45	tr	tr	0.70	0.16	1.25	17.3
24:0	4.45	18.7	1.1	11.81	6.28	8.89	30.8
25:0	0.33	2.4	1.4	1.61	0.99	0.69	5.9
26:0	7.06	37.6	17.5	11.11	9.10	6.53	9.2
27:0	1.84	1.7	0.71	1.52	0.75	1.21	2.6
28:0	8.50	21.2	49.5	25.14	12.75	9.53	6.3
29:0	0.31	1.3	0.51	0.10	0.20	tr	—
30:0	5.08	14.4	20.9	20.96	3.00	2.93	—
Phytol	55.84	—	nad	12.97	33.76	33.46	tr
Others	8.90	—	tr	3.76	27.67	24.29	—
Total absolute concn (μg/g dry wt.)	2270	—	—	150	1500	100	—

tr = Trace, <0.1%.
nad = Not accurately determined.
TSE = Total solvent extractables.

differs from the leaves where the ratio of sitosterol to stigmasterol is 3 to 1.

The distribution of alcohols in the leaves of *A. marina* (Table 3) is typical of most higher plants [8], ranging from 18:0 to 30:0 and maximizing at 28:0. Phytol was detected in significant quantity in both the fresh and dead leaf samples, but was not detected in the wax fraction of the fresh leaves. The alcohol fraction of the solvent extract of the fresh leaves contained a significantly higher relative and absolute amount of phytol than was found in the dead leaves indicating that rapid degradation of the phytol and the chlorin pigment occurs during leaf decay (equivalent chlorophyll-a ratio of fresh leaves/dead leaves is 35). The concentration of bound alcohols in the fresh leaves was appreciably lower than the level of solvent extractable alcohols (Table 4), and was not observed to increase on leaf decay. The distribution of alcohols within the pneumatophore sample was unusual, with little even over odd predominance occurring. Phytol was a minor component only of this fraction after saponification reflecting the low concentration of chlorophyll in the pneumatophores.

Six compounds identified by characteristic mass spectral fragmentation patterns and GC retention data as triterpene alcohols were found in both the fresh and dead leaves (Table 5) and in the pneumatophores of *A. marina* (these compounds were major components in the wax fraction of the leaves). The triterpene alcohols identified were: urs-12-ene-3β-ol, olean-12-ene-3β-ol, lup-20(29)ene-3β-ol and friedoolean-3β-ol. The major component (RR_t 1.168) has not yet been fully identified. The absolute amounts of this class of compound in both the fresh and dead leaf samples were similar, apart from a large decrease in the concentration of friedoolean-3β-ol. This constancy in absolute amounts of the triterpene alcohols between the two samples was also observed for the hydrocarbons, sterols and fatty alcohols indicating that these classes of compounds are not degraded rapidly during leaf decay. This may reflect their limited value as microbial nutrients.

The monobasic acid composition of the fresh leaves of *A. marina* (Table 6) ranges from C_{12} to C_{32} with 16:0, 18:1ω9*, 18:2ω6 and 18:3ω3 present as the predominant acids. These data are similar to those reported previously for *A. nitada* and *A. germinans* [2,3] but include the molecular structures of the component monocarboxylic acids unlike the previous work. The significance of knowing the isomer positions in the monoenoic acids has been documented previously [14,15], and the monoenoic acids detected in the fresh leaves were: 15:1, 16:1ω7, 16:1ω9, *trans* 16:1ω13, 17:1ω8, 18:1ω7, 18:1ω9, 19:1 and 20:1ω9.

The bound monobasic acids (released after saponification of the solvent extracted leaf residue) of the fresh

* Double bond positions (ω) are numbered from the methyl end of the fatty acid, all subsequent double bonds are methylene interrupted.

Table 4. Absolute amounts of lipid classes of *Avicennia marina*

Lipid class	Concentration μg/g (dry wt.)		
	Fresh leaves	Dead leaves	Ratio dead/fresh
Hydrocarbons	3070	3200	1.04
Sterols	1900	1640	0.86
Alcohols TSE	2270	1500	0.66
TSE less phytol	1000	990	0.99
Phytol	1270	510	0.40
Bound alcohols	150	100	0.66
Triterpene alcohols	1090	1130	1.04
Monobasic acids TSE	18420	7730	0.42
Saturated short-chain	5190	4040	0.78
Unsaturated	12660	3370	0.27
Long-chain	570	320	0.56
Bound	930	2520	2.71
Saturated short-chain	330	1140	3.45
Unsaturated	440	770	1.75
Long-chain	160	610	3.81
Total	19350	10250	0.53
Saturated short-chain	5520	5180	0.94
Unsaturated	13100	4140	0.32
Long-chain	730	930	1.27
α,ω-Dibasic acids	1600	2870	1.79
ω-Hydroxy acids	1190	1550	1.30

TSE = total solvent extractable.

leaves contained lower relative amounts of 18:2ω6 and 18:3ω3. This decrease is largely compensated for by a corresponding increase in the relative amount of high MW monobasic acids (22:0 to 30:0). The ratios of solvent extractable and bound long chain acids in dead to fresh leaves (Table 4) suggest some interconversion from solvent extractable to bound is occurring for this lipid class. A similar change is observed for the short chain (C < 22) fatty acids. The absence of long-chain acids in the solvent extracted fraction of the fresh leaves after the wax has been removed demonstrates their non-involvement in the membrane lipids.

The major difference between the monobasic acid composition of the fresh and dead leaves is the marked decrease in the relative and absolute amount of 18:2ω6 and 18:3ω3. The relative abundance of 14:0 and 16:0 increased dramatically. During leaf decay under oxic conditions all unsaturated acids present would be expected to be degraded after cellular lysis; the rate being dependent on the degree of unsaturation.

The presence of a number of *iso* and *anteiso* branched acids, 17:1ω10 and ω6, cyclopropyl 19:0 along with higher relative amounts of 14:0, 16:0, and 18:1ω7 in the dead leaves sample is indicative of a significant contribution to the component fatty acids by sedimentary microorganisms involved in leaf degradation. The bacterial origin of these acids has been reported previously [15], which suggests that significant bacterial colonization of the dead *A. marina* leaves has occurred. The longer chain monoenoic acids 22:1 and 24:1 not found in the fresh leaf sample and believed to be mainly the ω9-isomers were detected in the dead leaves of *A. marina*. The occurrence of these acids in sediments (thought to be of yeast origin) and *Mycobacteria* has been reported previously [16,17] indicating that an exogenous origin for these acids in the dead leaves of *A. marina* is probable.

The absolute amounts of the long-chain saturated fatty acids in the dead leaves is increased when compared with the fresh leaves (Table 4). These acids are probably less viable microbiological substrates than the short chain saturated and unsaturated fatty acids as noted previously [2,3]. The presence of a number of C_{20} and C_{22} polyunsaturated fatty acids not detected in the fresh leaves, but present in the dead leaves sample indicates that an alternative source for these acids is likely. Marine diatoms are rich in 20:4ω6 and 20:5ω3 [18], however, colonization by other microscopic algae e.g., certain Chrysophyta [19] could also account for these acids. The fatty acid distribution of the pneumatophores is similar to the fresh mangrove leaves although the relative concentration of 18:3ω3 in particular is reduced. Small quantities of 22:1 and 24:1 were also detected.

The α,ω-dibasic acid composition of *A. marina* isolated by saponification of the leaf residues after solvent extraction (Table 7) shows di 16:0, di 18:0 and di 18:1 as the major components with di 18:2 present at a lower relative level. The absolute amount of the α,ω-dibasic acids in the fresh leaves is 8% of that of the monobasic fatty acids. The dead leaves sample showed a similar diacid distribution to that of the fresh leaves of *A. marina* with some changes in the minor constituents observed. The relative amount of di 18:2 was diminished and the higher MW components increased. The absolute amounts of the diacids in the dead leaves were 1.8 times that observed in the fresh leaves and 28% of that of the monobasic acids in the dead leaves. It was also noted that the absolute amount of long-chain monoacids in the dead

Table 5. Triterpene alcohol concentration of the fresh and dead leaves of *Avicennia marina*

Compound	RR_t*	Percentage composition Fresh leaves	Dead leaves	M^+	MS fragmentation *m/e* (rel. int.) (of TMSi ether)
Unidentified A	0.904	1.6	2.7	No mass spectral data.	
Lup-20(29)ene-3β-ol (lupeol)	0.937	3.6	4.9	498(17)	189(100), 205(90), 190(86), 218(80), 203(75), 109(74), 147(26), 119(22), 161(20), 174(20), 279(20), 229(18), 293(8), 257(6).
Olean-12-en-3β-ol (β-amyrin)	1.000	11.9	12.1	498(1.3)	218(100), 203(35), 190(19), 189(17), 204(12), 109(7), 121(7), 135(7), 147(7), 175(7), 161(4), 257(3), 279(3), 483(1).
Urs-12-en-3β-ol (α-amyrin)	1.110	17.6	20.5	498(3)	218(100), 189(22), 190(16), 203(16), 220(16), 135(12), 121(10), 109(8), 147(7), 161(6), 175(5), 279(5), 257(1), 408(1).
Unidentified B	1.168	57.6	57.1	498(19)	189(100), 109(73), 190(64), 135(58), 203(51), 121(50), 218(32), 147(29), 175(28), 156(27), 369(21), 231(16), 279(15), 393(10), 257(9), 407(8), 299(7), 483(6), 245(5), 325(4).
Friedoolean-3β-ol (friedelinol)	1.391	7.7	2.7	500(2.5)	69(100), 109(70), 123(68), 125(62), 205(42), 273(37), 163(33), 137(32), 159(30), 190(26), 179(25), 149(23), 246(21), 231(18), 302(16), 217(15), 426(15), 341(11), 287(6), 259(5), 411(5).
Total absolute concn (μg/g dry wt.)		1090	1130		

* RR_t on SE30, β-Amyrin = 1.0.

leaves was increased by a factor of 1.3 compared with the fresh leaves (Table 4), this factor being the same as that observed for the total ω-hydroxy acids. The high absolute concentrations of α,ω-dibasic acids in *A. marina* is unusual since these acids are generally considered to be minor constituents of leaf cutin [20]. The pneumatophores contained the same diacid components as the *A. marina* leaves although di 18:2 was not detected. The dibasic acid concentration in the pneumatophores was calculated, however, to be 1.7 times the monobasic acid concentration, significantly different from that found in the leaves.

The ω-hydroxy acid profile of the fresh and dead leaves of *A. marina* (Table 8) shows both identical chain-length distribution and similar relative concentrations of individual components when compared with the α,ω-dibasic fatty acids of *A. marina* (Table 7). These data suggest the direct biosynthesis of the α,ω-dibasic acids from the ω-hydroxy acids in the mangrove *A. marina*. This hypothesis has been reported previously [21], as has the oxidative conversion of monobasic acids to ω-hydroxy acids [22].

The total absolute amounts of monobasic acids in the dead leaves of *A. marina* are 53% of the absolute amounts found in the fresh leaves, whereas the absolute amounts of both the α,ω-dibasic and ω-hydroxy acids have increased (Table 4). This observed increase in the absolute level of diacids in the dead leaves indicates preferential degradation of the cellular contents and resultant enrichment of the cutin (obtained by saponification of the leaf residues after solvent extraction) and wax derived components.

The chain length distribution of the α,ω-dibasic acids and ω-hydroxy acids in the pneumatophores of *A. marina* (Tables 7 and 8) are very similar, as expected. The presence of ω-hydroxy 12:0 and 14:0 in the pneumatophores but not in the leaf suggests some differences in the chain-length specificities of the enzymes involved. These shorter chain-length ω-hydroxy acids are not further oxidized to α,ω-dibasic acids. We suggest that the ω-hydroxy acid dehydrogenase is specific for the C_{16} and C_{18} chain lengths in the pneumatophores of *A. marina*.

The data presented on the lipid distribution in the decayed leaves relative to the fresh leaves of *A. marina* (Table 4) show the only significant changes to be a reduction in the total amount of monobasic acids present

Table 6. Monobasic acid composition of *Avicennia marina*

		Percentage of total monoacids						
		Fresh leaves				Dead leaves		
Homologue	ECL*	TSE†	Wax	(TSE − wax)	Bound	TSE	Bound	Pneumatophores
12:0	12.00	0.10	1.0	2.8	0.15	0.27	0.80	0.27
12:1	12.15	—	—	—	—	—	0.05	—
13:0	13.00	—	—	tr	—	tr	—	—
14:0	14.00	0.82	1.1	8.4	0.97	9.09	5.82	3.8
14:1	14.28	—	—	—	tr	—	0.07	—
iso 15:0	14.57	—	—	—	—	0.17	0.19	—
anteiso 15:0	14.70	—	—	—	—	0.26	0.31	—
15:0	15.00	0.08	0.28	1.0	0.15	0.36	0.29	0.08
15:1	15.17	0.08	tr	tr	0.16	1.35	0.77	1.3
iso 16:0	15.56	—	—	—	—	—	0.20	—
16:0	16.00	21.88	3.9	21.8	22.36	31.41	24.71	17.1
16:1ω9	16.25	0.08	—	—	0.08	0.26	0.20	tr
16:1ω7	16.33	1.03	0.21	2.1	3.57	3.55	1.56	0.44
trans 16:1ω13	16.42	1.03	—	nad	0.17	0.12	0.13	tr
16:1ω5	16.44	—	—	—	—	0.05	0.07	—
iso 17:0	16.54	—	—	—	—	tr	—	—
anteiso 17:0	16.68	—	—	—	—	tr	—	—
16:2ω4	16.93	0.08	—	—	0.05	tr	tr	—
17:0	17.00	0.30	1.1	0.90	0.22	0.67	0.57	0.27
17:1ω10	17.22	—	—	—	—	0.12	—	—
17:1ω8	17.29	0.05	0.25	tr	tr	0.16	0.20	0.08
17:1ω6	17.38	—	—	—	—	tr	tr	—
18:0	18.00	3.65	1.7	1.5	5.35	6.30	5.49	2.1
18:1ω9	18.27	8.95	1.1	4.8	10.69	8.52	9.34	12.6
18:1ω7	18.35	0.32	tr	tr	0.25	2.06	3.04	1.1
18:2ω6	18.79	22.15	0.29	23.8	17.88	12.69	10.09	36.2
19:0	19.00	0.11	0.05	tr	0.14	0.26	0.27	0.17
18:3ω6	19.09	—	—	—	—	0.05	—	—
cyclo ∇19:0	19.29	—	—	—	—	0.30	0.17	—
19:1	19.31	0.06	—	—	0.06	0.07	0.21	—
18:3ω3	19.42	34.66	tr	32.4	14.32	13.56	3.29	4.9
20:0	20.00	1.00	3.9	0.5	4.16	2.01	5.10	0.82
20:1ω9	20.26	0.31	1.2	nad	0.44	0.24	0.32	0.17
21:0	21.00	0.06	0.28	—	0.45	0.23	0.78	0.16
20:4ω6	21.34	—	—	—	—	0.31	—	—
20:4ω4	21.70	—	—	—	—	tr	—	—
20:4ω3	21.76	—	—	—	—	tr	—	—
20:5ω3	21.98	—	—	—	—	0.43	—	—
22:0	22.00	0.17	2.9	—	3.66	0.36	4.18	1.3
22:1ω9	22.25	—	—	—	—	—	0.14	0.7
23:0	23.00	0.05	0.61	—	0.47	0.10	0.94	0.22
24:0	24.00	0.44	21.9	—	7.71	0.53	10.53	4.6
24:1ω9	24.17	—	—	—	—	0.25	1.11	0.74
22:6ω3	24.37	—	—	—	—	0.20	—	—
25:0	25.00	0.26	4.3	—	0.45	0.26	0.87	0.49
26:0	26.00	0.91	36.3	—	3.92	0.71	6.00	4.5
27:0	27.00	0.06	1.4	—	—	0.06	0.11	0.22
28:0	28.00	0.59	12.5	—	0.52	0.72	1.16	3.3
29:0	29.00	tr	0.45	—	—	0.08	tr	0.08
30:0	30.00	0.31	2.7	—	1.52	0.84	0.42	0.78
31:0	31.00	tr	—	—	—	tr	—	—
32:0	32.00	0.30	—	—	—	0.44	—	—
Unidentified		0.28	0.64	tr	0.13	0.72	0.52	1.5
Total absolute concn μg/g (dry wt.)		18420	—	—	930	7730	2520	—

* Equivalent chain length.
† TSE = Total solvent extractable.
tr = trace <0.05%.
nad = Not accurately determined.

Table 7. α,ω-Dibasic acid composition of *Avicennia marina*

Homologue	Percentage of total diacids		
	Fresh leaves	Dead leaves	Pneumatophores*
9:0	—	0.57	tr
10:0	—	0.29	—
11:0	—	tr	—
14:0	tr	tr	—
15:0	tr	tr	—
16:0	31.44	29.76	26.9
16:χ	0.66	0.63	—
17:0	0.33	0.31	0.1
18:0	10.49	10.69	11.7
18:1	55.39	56.49	58.2
18:2	1.31	0.23	—
20:0	0.24	0.63	—
22:0	tr	0.10	0.1
24:0	—	0.10	—
Total absolute concn μg/g (dry wt.)	1600	2870	—

tr = Trace, <0.1%.

* An additional unidentified component (*ca* 3%) was observed eluting after the 18:0 diacid.

Table 8. ω-Hydroxy acid composition of *Avicennia marina*

Homologue	Percentage composition of total ω-hydroxy acids		
	Fresh leaves	Dead leaves	Pneumatophores
12:0	—	—	4.8
14:0	—	—	9.6
16:0	38.5	31.3	11.3
18:0	5.4	10.0	1.8
18:1	53.5	58.0	72.5
20:0	1.8	0.4	—
22:0	0.4	0.3	—
24:0	0.2	tr	—
Total absolute concn μg/g (dry wt.)	1190	1550	—

tr = Trace, <0.1%.

(due largely to a decrease in concentration of the C_{18} polyunsaturated fatty acids) and a resultant enhancement of the concentrations of α,ω-dibasic acids, ω-hydroxy acids and long chain monobasic acids. These data suggest preferential preservation of the cutin-derived acids, relative to the cellular and wax derived lipids. This resistance to degradation should allow the cutin acids to be used as quantitative markers of the input of *Avicennia marina* to the mangrove associated sediments at Port Franklin. Our data also point to the relative importance of cutin acids as significant original inputs to mangrove peats, and indeed to many fossil fuel deposits.

EXPERIMENTAL

The *A. marina* samples were collected at Port Franklin, Victoria (lat. 38°46'S. Long 146°20'E.). Prior to extraction of lipid material all samples were washed thoroughly with dist. H_2O to remove any sediment and adhering detritus.

Lipid classes discussed were isolated using techniques previously described [7,14,15,23]. The epicuticular wax fraction analysed was obtained by dipping the fresh leaves in $CHCl_3$ for 1 min [24]. GC analysis of samples was performed on a glass SCOT SE30 60 m × 0.5 mm i.d. column. Mono- and α,ω-dibasic acid Me esters were also analysed using a glass WCOT

SIL47CNP (47% cyanopropyl silicone) 43 m × 0.2 mm i.d. column [25]. He was used as carrier gas (linear flow: 20 cm/sec). The SE30 column was programmed from 160° to 280° at 2.5° per min and the SIL47CNP column from 100° to 240° at 3° per min. FID detectors were used with injector and manifold temperatures at 280° and 300° respectively. Monobasic Me esters were tentatively identified by co-chromatography with authentic standards and by RR_t [26, 27] and ECL measurements [28, 29]. All lipid components were quantitated by calibrated GC response and are subject to errors of ±10%.

Sterol identifications were based on RR_t co-injection with authentic standards and OsO_4 oxidation [25]. The latter technique allowed differentiation of the degree of unsaturation of individual sterols. This is especially important in the separation of 28-isofucosterol and 5α-stigmastanol which had the same R_t on the GC columns employed. The major sterols were confirmed by GC–MS.

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