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LIPID COMPONENTS OF THE SEAGRASSES *POSIDONIA AUSTRALIS* AND *HETEROZOSTERA TASMANICA* AS INDICATORS OF CARBON SOURCE*

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Key Word Index—*Posidonia australis*; Posidoniaceae; *Heterozostera tasmanica*; Zosteraceae; seagrasses; lipids; hydrocarbons; sterols; monocarboxylic acids; α, ω-dicarboxylic acids; ω-hydroxy fatty acids; chemical marker; food chain.

Abstract—The component hydrocarbons, sterols, alcohols, monocarboxylic, α, ω-dicarboxylic and ω-hydroxy acids of the seagrasses *Posidonia australis* and *Heterozostera tasmanica* and a sample of *P. australis* detritus are reported. The fresh leaves of *P. australis* and *P. australis* detritus are characterized by a distinctive distribution of solvent-extractable long-chain monocarboxylic, α, ω-dicarboxylic and ω-hydroxy acids. This distinctive pattern should enable these lipid components along with other distinctive components to be used as chemical markers of the seagrass *P. australis*. *H. tasmanica* is characterized by (1) higher relative concentrations of 16:2ω6 and 16:3ω3 than *P. australis*, (2) the absence of the distinctive distribution pattern of long-chain monocarboxylic and ω-hydroxy acids observed for *P. australis*, (3) the absence of α, ω-diacids and (4) a lower absolute concentration of ω-hydroxy acids than *P. australis*.

INTRODUCTION

Seagrass meadows provide a unique environment which trap terrestrially derived inputs and produce significant quantities of materials of plant and animal origin which are known to support microorganisms and phytoplankton [1]. These materials include whole leaf and rhizome material, particulate detritus and dissolved organic matter. Apart from their role in providing materials for many marine food chains, seagrasses also provide a physical support for numerous epiphytic organisms [2]. The importance of seagrass meadows in providing a habitat for the growth of fish and invertebrates, including commercial species, has also been recognized [1].

Our laboratories are examining food chain structure and geochemical characteristics of seagrass communities using techniques which include biochemical analysis of the digestion of seagrasses, microscopic and macroscopic examination of gut contents, stable carbon isotope determinations and the analysis of various lipid classes present in the seagrass and associated organisms for use as chemical markers.

The concept of biological marker is not a new one [3, 4] and involves the use of specific lipid components in tracing the origin of biological material. Although this term is more commonly used in geochemical studies it can also be applied to marine food chain studies where the use of a chemical marker may be more appropriate. An evaluation of lipid data of seagrass species in the literature revealed the paucity of such reports. Although reports exist on individual lipid classes including fatty acids [5], hydrocarbons [6, 7] and sterols [8], none include data on the broad spectrum of lipid classes present in a particular seagrass species. These component lipids may have the potential to be used as 'chemical markers'.

In this report a detailed lipid analysis of the fresh leaves from two marine seagrasses; *Posidonia australis* and *Heterozostera tasmanica* is presented. A sample of *P. australis* detritus has also been analysed. This sample included encrusting epiphytic material which may be important as a carbon source in marine food chains. Observed changes in lipid composition with leaf decomposition are also discussed. Lipid classes analysed included hydrocarbons, sterols, monocarboxylic fatty acids, α,ω-dicarboxylic fatty acids, ω-hydroxy acids and chlorophyll pigments. The potential of these lipid components for use as chemical markers is examined for the two seagrasses analysed. Previous work by our group has shown the interpretative usefulness of further fractionation of the fatty acids and alcohols into solvent extractable and bound (obtained by base saponification of the solvent extracted residue) fractions [9]. The exact molecular structure of component monocarboxylic fatty acids is reported for each seagrass sample analysed, since such data are essential for both biochemical and geochemical interpretation of the components present [9, 10].

*Part 1 in the series "Study of Food Chains in Seagrass Communities".

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RESULTS AND DISCUSSION

Quantitative lipid composition data on the marine seagrasses *Posidonia australis*, *Heterozostera tasmanica* and a sample of *P. australis* detritus are reported in Table 1. Monocarboxylic fatty acids liberated from acyl lipids were the major lipid class present in both seagrasses and in the *P. australis* detritus sample. Other lipid classes found at high abundance in both *P. australis* and *H. tasmanica* included sterols and chlorophyll pigments. The concentration of chlorophyll in *P. australis* detritus was greatly reduced when compared with the fresh leaves. Previous studies by our group and others have shown that considerable chlorophyll breakdown occurs during leaf senesence [11, 12]. Chlorophyll *a* : *b* ratios of 1.8 and 1.5 were observed for *P. australis* and *H. tasmanica*, respectively.

High concentrations of α, ω-dicarboxylic and ω-hydroxy acids were found in *P. australis*. *H. tasmanica* contained a significantly lower abundance of ω-hydroxy acids than *P. australis* and α, ω-diacids were not detected. The significance of these components will be discussed later. *P. australis* detritus lipid class abundances were generally at lower absolute levels compared with the fresh *P. australis* leaf sample. It is worth noting, however, that the absolute amounts of solvent-extractable α, ω-dicarboxylic fatty acids and bound monocarboxylic fatty acids showed smaller decreases in concentration than the other lipid classes, indicating that these more persistent components may be useful as chemical markers for *P. australis*.

The component hydrocarbons of *P. australis* and *H. tasmanica* consist exclusively of *n*-alkanes maximizing at *n*-C_{19} and *n*-C_{17}, respectively (Table 2). *H. tasmanica* contained a wider range, *n*-C_{15}–*n*-C_{33}, than did *P. australis*. The maximum for *Zostera marina* has been shown to vary seasonally, with a lower maximum corresponding to the flourishing stage [7]. The two samples analysed in this report were collected within 2 months of each other, thus the observed differences in C_{max} of these two seagrasses are probably due to actual species differences rather than metabolic adjustments within these plants. Odd chain length alkanes represented greater than 80% of the total alkanes for both seagrasses, which is typical of the alkane distribution found in plant waxes [13]. However, the low abundance of high MW alkanes ($> C_{20}$) and the abundance of *n*-C_{17} and *n*-C_{19} is more typical af algal hydrocarbons [14]. The similar distributions of the *n*-alkanes in these two seagrasses and in epiphytic algae collected from *P. australis* [15] suggests that *n*-alkanes will probably not be useful as specific chemotaxonomic markers for *P. australis* and *H. tasmanica*.

The major sterols identified in *P. australis* and *H. tasmanica* at similar relative and absolute concentrations were; 24 - ethylcholest - 5 - en - 3β - ol, 24 - ethylcholesta - 5,22*E* - dien - 3β - ol and 24 - methyl - cholest - 5 - en - 3β - ol (Table 3). Cholest - 5 - en - 3β - ol and 5α-stanols were detected in both seagrasses at very low abundance. The finding of 5α-stanols is interesting in view of the generally low abundance of stanols in nature. The *P. australis* detritus sample

Table 1. Lipid composition of fresh *P. australis*, *P. australis* detritus and *H. tasmanica*

	Composition μg/g (dry) of tissue		
	P. australis (live fronds)	*P. australis* (detritus)	*H. tasmanica*
Solvent-extractable lipids			
Total lipid	76300 (7.6%)	12700 (1.3%)	38900 (3.9%)
Chlorophyll	6700	140*	5500
Chlorophyll *a* : *b* ratio	1.8	ND	1.5
Hydrocarbons	28	ND	150
Sterols	1800	230	1900
Phytol	2200	11	1300
Monocarboxylic acids	13500 (1.3%)	2200 (0.22%)	11200 (1.1%)
α, ω-Dicarboxylic acids	456	210	ND
ω-Hydroxy acids	3450	240	150
Bound lipids			
Phytol	8	7	35
Monocarboxylic acids	620	330	2300
Monocarboxylic acid/sterol ratio	7.5	9.6	5.9
Monocarboxylic acid/ω-hydroxy acid ratio	4.0	9.1	74.7
Monocarboxylic acid/total lipid ratio	0.18	0.17	0.29

*Includes chlorophyll degradation products.
ND—Not detected.

Table 2. Hydrocarbon composition of fresh *P. australis* and *H. tasmanica*

Hydrocarbon	Percentage composition *P. australis*	*H. tasmanica*
15:0	17.0	8.0
16:0	3.2	2.6
17:0	17.1	45.2
18:0	5.6	1.9
19:0	27.3	12.9
20:0	2.2	0.9
21:0	11.6	5.1
22:0	2.4	1.4
23:0	3.6	1.6
24:0	2.6	1.4
25:0	7.4	2.6
26:0	TR	2.0
27:0	TR	2.9
28:0	TR	1.9
29:0	—	2.7
30:0	—	2.5
31:0	—	1.9
32:0	—	1.4
33:0	—	0.7
Total absolute concn μg (dry wt)	2.8	15.0

TR—trace, < 0.1%.

analysed differed quite markedly from the fresh seagrasses analysed, in that cholest - 5 - en - 3β - ol was the predominant sterol detected, and significant levels of cholesta - 5 - 22*E* - dien - 3β - ol and 24 - methylcholesta - 5, 24(**28**) - dien - 3β - ol were also present. All the sterols detected in the *P. australis* detritus at increased levels relative to the living *P. australis* sample have been previously reported by our group as significant contributors to the component sterols of a mixed plankton sample [16]. The low relative abundances of the 5α-stanols; 5α - cholestan - 3β - ol and 24 - ethyl - 5α - cholestan - 3β - ol in *P. australis* detritus is also similar to that observed in the plankton sample [16]. Thus, it appears that these components are not of a seagrass origin, but originate from the epiphytic material found associated with the *P. australis* detritus. It is also worth noting that the major sterols in the *P. australis* leaf sample, in particular 24 - ethylcholest - 5 - en - 3β - ol were detected at significantly lower relative and absolute amounts in the *P. australis* detritus sample. These data are consistent with a rapid degradation of the seagrass sterols upon leaf senesence. Thus, although the input of fresh seagrass material into either a sediment or a marine food chain could readily be quantified using sterols as marker components, an accurate absolute quantitation of seagrass debris material would be difficult since the extent of seagrass decay would be an important variable in such estimations.

The monocarboxylic fatty acid components of *P. australis*, *H. tasmanica* and *P.* australis detritus are presented in Table 4. The predominant solvent-extractable monoacids in *P. australis* in decreasing absolute abundance were 18:3ω3* 16:0, 18:2ω6, *trans* 16:1ω13, 18:0, 26:0 and 28:0. These components accounted for greater than 90% of the solvent-extractable monoacids. This laboratory has previously reported on the significance of monoenoic isomer position [17, 18] and the monoenoic acids found in *P. australis* were 16:1ω9, 16:1ω7, *trans* 16:1ω13, 17:1ω8, 18:1ω9, 18:1ω7, 20:1ω9 and

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

Table 3. Sterol composition of fresh *P. australis*, *P. australis* detritus and *H. tasmanica*

*RR**†	Sterol	Percentage composition *P. australis* (live fronds)	*P. australis* (detritus)	*H. tasmanica*
0.895	Cholesta-5, 22*E*-dien-3β-ol	—	2.3	—
1.000	Cholest-5-en-3β-ol	0.4	38.7	0.7
1.030	5α-Cholestan-3β-ol	—	2.6	—
1.092	Cholesta-5, 24-dien-3β-ol	—	0.4	—
1.115	24-Methylcholesta-5, 22*E*-dien-3β-ol	—	0.7	—
1.151	5α-Cholest-7-en-3β-ol	—	0.3	—
1.282	24-Methylcholesta-5, 24(28)-dien-3β-ol	—	2.0	—
1.313	24-Methylcholest-5-en-3β-ol	5.4	3.4	9.0
1.417	24-Ethylcholesta-5, 22*E*-dien-3β-ol	9.6	10.8	11.0
1.467	24-Ethyl-5α-cholest-22*E*-en-3β-ol	0.2	1.0	0.4
1.630	24-Ethylcholest-5-en-3β-ol	83.7	20.7	76.4
1.679	24-Ethyl-5α-cholestan-3β-ol	—	4.5	0.1
	Unidentified	1.4	12.7	1.5
	Total absolute concentration μg/g (dry wt)	1790	230	1870

*Using a SE30 capillary column.

Table 4. Monocarboxylic acid composition of fresh *P. australis*, *P. australis* detritus and *H. tasmanica*

Acid	ECL*	Percentage composition					
		P. australis (live fronds)		*P. australis* detritus		*H. tasmanica*	
		Solvent-extractable	Bound	Solvent-extractable	Bound	Solvent-extractable	Bound
12:0	12.00	0.16	0.22	—	2.8	—	0.23
13:0	13.00	0.01	—	—	0.32	—	—
iso 14:0	13.54	—	—	0.38	0.38	—	—
14:0	14.00	0.61	3.2	3.5	6.5	1.8	0.74
14:1ω7	14.19	—	—	0.10	0.12	0.51	0.04
14:1ω5	14.32	—	0.02	0.26	0.26	—	—
iso 15:0	14.52	—	0.04	1.5	1.8	0.02	0.04
anteiso 15:0	14.66	—	0.05	0.66	0.41	0.02	0.05
15:0	15.00	0.09	0.44	1.2	1.24	0.38	0.61
15:1ω8	15.18	—	0.08	—	0.14	0.03	0.03
15:1ω6	15.27	—	—	—	0.10	0.02	0.04
iso 16:0	15.52	0.13	—	0.26	0.23	0.07	0.01
16:0	16.00	25.5	40.3	43.9	58.1	33.0	38.1
16:1ω9	16.15	0.09	0.30	0.68	0.82	0.42	0.56
16:1ω7	16.21	0.39	0.15	3.5	1.1	1.6	0.76
16:1ω5	16.32	—	—	0.38	1.0	0.04	0.03
trans 16:1ω13	16.49	2.6	1.4	0.32	1.1	1.8	2.7
iso 17:0	16.52	—	0.27	0.51	0.08	—	—
16:2ω6	16.56	—	—	—	—	0.37	0.36
anteiso 17:0	16.66	—	0.02	0.04	0.23	—	—
16:2ω4	16.76	0.03	—	—	—	0.07	0.07

16;3ω6	16.82	—	—	—	0.08	—	0.13
17:0	17.00	0.31	0.31	0.67	0.68	0.86	1.3
16:3ω3	17.16	—	—	—	—	3.1	2.5
17:1ω8	17.18	0.07	—	0.04	0.16	—	—
iso 18:0	17.51	—	—	—	—	—	0.04
18:0	18.00	1.9	4.6	5.7	5.6	2.8	3.4
18:1ω9	18.16	1.1	0.92	3.5	1.9	2.4	1.5
18:1ω7	18.23	0.26	0.18	7.4	2.1	0.73	0.54
18:1ω5	18.36	—	0.10	0.19	2.9	0.02	0.03
18:2ω6	18.63	10.4	11.9	1.7	2.9	18.7	14.7
18:3ω6	18.88	0.02	0.03	0.44	0.24	0.07	0.02
19:0	19.00	—	0.04	—	0.23	—	0.26
18:3ω3	19.26	51.6	33.4	0.74	2.2	28.1	25.2
20:0	20.00	0.15	0.34	0.53	0.44	0.48	1.07
20:1ω9	20.16	0.05	—	—	—	0.07	0.11
20:1ω7	20.29	0.04	0.16	1.22	0.16	0.07	0.08
21:0	21.00	0.07	0.04	0.08	0.04	0.02	0.25
20:4ω6	21.05	—	—	1.0	0.11	0.08	TR
20:5ω3	21.65	0.07	0.05	TR	0.23	0.13	0.05
22:0	22.00	0.19	0.15	0.27	0.29	0.46	1.2
23:0	23.00	0.15	TR	0.09	0.08	0.07	0.28
24:0	24.00	0.37	0.16	1.1	0.19	0.71	0.73
25:0	25.00	0.05	0.03	0.21	0.02	0.10	0.05
26:0	26.00	1.5	0.05	7.4	0.20	0.22	0.05
27:0	27.00	0.05	—	0.44	TR	0.01	TR
28:0	28.00	1.3	0.03	9.6	0.06	0.08	0.01
Unidentified		0.77	1.02	0.47	2.46	0.54	2.18
Total absolute concn (μg/g) dry wt		13500	620	2200	330	11200	2300

*ECL/equivalent chain length, SP1000 capillary column.
TR—trace, < 0.01%.

20:1ω7. The Δ^{9}* isomers are typical of higher plants; 16:1ω7 and 18:1ω9 were the major Δ^{9} isomers present. The only *trans* monounsaturated acid found in *P. australis* is *trans* 16:1ω13. This acid is widely distributed in higher plants and other photosynthetic organisms and originates in the chloroplast [19, 20]. The small amount of vaccenic acid (18:1ω7) detected in *P. australis* is noteworthy although oleic acid (18:1ω9), the common plant isomer, predominates. This report of vaccenic acid in seagrass lipids further supports the wider occurrence of this acid [17, 21, 22] than has been previously recognized. In view of the predominance of the Δ^{9} isomers, it seems probable that the Δ^{9}-desaturase in *P. australis* acts on both 16:0 and 18:0 and that a small proportion of Δ^{9}-16:1 is chain elongated, to Δ^{11}-18:1.

The bound monocarboxylic acids (Table 4) (obtained after base saponification of the solvent-extracted residue) of *P. australis* contained similar relative amounts of all the monoacids found in the solvent-extractable fraction with the exception of the long-chain monoacids 26:0 and 28:0 which were detected at lower relative levels. An analysis of the leaves of the mangrove *Avicennia marina* by our laboratory has in contrast noted the presence of these long-chain acids as major components in the cutin fraction (released by base saponification of the solvent-extracted residue) of the leaves [11]. The bound lipid data presented in this report may provide useful information should it be found that the solvent-extractable lipids are preferentially metabolized during seagrass digestion. Analysis of the bound lipid components along a digestive tract may therefore reveal which ingested material is not being metabolized and/or assimilated by the feeding animal.

When compared with fresh *P. australis* leaves, the solvent-extractable monoacid components from *P. australis* detritus are characterized by significantly lower relative levels of the major unsaturated components found in the fresh leaves of *P. australis*; 18;2ω6, 18:3ω3 and *trans* 16:1ω13.The reduction in the total amount of monocarboxylic fatty acids is largely due to a decrease in concentration of the C_{18} polyunsaturated fatty acids as was observed for the mangrove *A. marina* [11]. During leaf decay, the unsaturated acids present would be expected to be degraded after cellular lysis. The presence of the monoacids previously reported to be of bacterial origin [18]:15:0, 15:1 and 17:1ω6, and ω8 isomers, 18:1ω7, *iso* and *anteiso* branched chain fatty acids, at higher relative concentration in the *P. australis* detritus indicates that significant bacterial colonization has occurred on the dead *P. australis* leaves. The relative amounts of the long-chain, solvent-extractable, saturated, even-carbon numbered monoacids (22:0–28:0) are also markedly increased in the *P. australis* detritus sample, although the absolute amounts of these compounds in both fresh *P. australis* and *P. australis* detritus samples are similar. An unusual distribution pattern of these long-chain monoacids exists in these two samples whereby an envelope distribution is not observed. Rather a stepwise increase of the even-chain components from 22:0 to 28:0 exists. The observed preservation of these solvent-extractable long-chain monoacids, along with their distinctive distribution pattern, may well allow these components to be used as quantitative markers of *P. australis* in seagrass associated sediments and in marine food chains. The distinctive distribution patterns also allow *P. australis* and *H. tasmanica* to be readily distinguished. The presence of a number of C_{18} and C_{20} polyunsaturated mono acids, which are acids that normally degrade rapidly, at increased relative concentration in the *P. australis* detritus when compared with the fresh *P. australis* leaves indicates that an alternative source to seagrass exists for these acids. Microscopic algae, many of which are high in the polyunsaturated fatty acids [4], are the probable source.

The bound monoacids of *P. australis* detritus were similar in distribution to the solvent-extractable components. It is of note, however, that the long-chain components in *P. australis* detritus were at low relative and absolute concentrations, indicating that monoacids are not being converted from solvent-extractable to bound lipids during seagrass decay. We have, however, previously observed this transformation for the long-chain monoacids of *A. marina* during leaf decay [11]. This result may be indicative of the different roles of these monoacid components in these two plants,

The solvent-extractable monoacids of *H. tasmanica* (Table 4) are characterized by the high abundances of 16:0, 18:3ω3, 18:2ω6, 16:3ω3, *trans* 16:1ω13 and 16:1ω7. This distribution is similar to that observed in most terrestial plants [23, 24] although the high relative levels of the polyunsaturated components; 16:3ω3 and 16:2ω6 is unusual. These acids have been reported previously in the seagrass *Zostera muelleri* by our group [25, 26] and may prove to be, when coupled with other lipid components, markers for these two seagrasses.

Long-chain monoacids (22:0 to 28:0, maximizing at 24:0) were found to be minor components (less than 1.5%) of the total monoacids in both the solvent-extractable and bound lipid fractions. The observed lower abundance of long-chain monoacids in *H. tasmanica* than in *P. australis* coupled with the data that *P. australis* also contains significantly greater amounts of α, ω-dicarboxylic and ω-hydroxy acids, which are both found typically in higher plant cutin material, is probably indicative of a poorly developed cuticle in *H. tasmanica*. This is one significant chemical differentiation observed between these two seagrasses.

The solvent-extractable α, ω-dicarboxylic acid components of fresh *P. australis* leaves and *P. australis* detritus are reported in Table 5. The similar diacid distribution found in both samples is rather unusual in that 16:0, 18:0 and 18:1, the diacids which usually acount for the majority of α, ω-dicarboxylic acids found in the cutin and suberin of higher plants, are absent. These cutin and suberin components of *A. marina* were not present as solvent-extractable components, but rather were released after base saponification of the solvent-extracted residue [11]. Further study is necessary to determine

*Δ^{9} designates the delta nomenclature system where double bonds are designated from the carboxyl end of the molecule.

Table 5. α, ω-Dicarboxylic acid composition of fresh *P. australis* and *P. australis* detritus

Diacid	Percentage composition	
	P. australis (live fronds)	*P. australis* (detritus)
24:0	6.6	5.8
25:0	—	—
26:0	32.5	24.2
27:0	1.5	—
28:0	59.4	70.0
Total absolute concn (μg/g dry wt)	450	210

the biological function of α, ω-diacids in *P. australis*. The unusual distribution pattern of diacids parallels that of the long-chain solvent-extractable monoacids; i.e. ranging from 24:0 to 28:0, predominantly even-carbon numbered in distribution and with a stepwise increase observed with increasing MW.

The distinct distribution pattern and the high absolute abundance of diacids in fresh *P. australis* leaves and *P. australis* detritus should enable these components to be used as markers of *P. australis* in our future studies. α, ω-Dicarboxylic acids were not detected in the sample of *H. tasmanica* analysed.

The solvent-extractable ω-hydroxy acid profiles of *P. australis*, *P. australis* detritus and *H. tasmanica* are presented in Table 6. The *P. australis* fresh frond and *P. australis* detritus samples show identical chain length distribution, 24:0–28:0 with the even carbon number components predominating, and similar relative amounts of individual components when compared with the long-chain solvent-extractable monocarboxylic and α, ω-dicarboxylic acids of *P. australis* (Table 5). These data suggest that oxidative conversion of monocarboxylic fatty acids to ω-hydroxy acids and the conversion of the ω-hydroxy acids to α, ω-dicarboxylic fatty acids is occurring in the seagrass *P. australis*. These interconversions have been reported previously in higher plants [27, 28]. Although α, ω-diacids and ω-hydroxy acids have been found in the cutin polyester structure [13] the nature of these compounds in *P. australis* are distinctive: (1) both of these lipid classes are contained in the solvent extract fraction as distinct from other higher plants where base saponification is necessary for the release of these compounds; (2) the chain length distribution which is not that normally found in terrestrial plants [13]. The distinctive distribution pattern of the long-chain mono-, α,ω-di- and ω-hydroxy carboxylic acids should enable these compounds along with other distinctive lipid components to be used as chemical markers of the seagrass *P. australis*.

The extent, however, to which these ω-hydroxy acids can be used as chemical markers for *P. australis* may be limited since the absolute concentration of these components in the *P. australis* detritus sample is only 7% of the high concentration found in the fresh *P. australis* leaves (3450 ppm). However, the distinct distribution of these acids should at least allow the presence of *P. australis* to be detected.

The solvent-extractable ω-hydroxy acid distribution of *H. tasmanica*, 20:0–28:0 with even carbon numbered components predominating, is similar to the long-chain monoacids detected in this seagrass although the maximum has shifted from 24:0 to 22:0. The absolute concentration of ω-hydroxy acids in *H. tasmanica* is significantly lower than that in *P. australis*, indicating, as discussed previously, a poorly developed cuticle in this seagrass. As was observed for the α, ω-diacids of *P. australis*, no 16:0, 18:0 or 18:1 ω-hydroxy acids were detected. These data indicate the different nature of the leaf structure in water-submerged seagrass when comparisons with terrestrial plants are undertaken.

This report provides much needed base-line lipid composition data of two seagrass species. The application of these data in marine food-chain and geochem-

Table 6. ω-Hydroxy acid composition of fresh *P. australis*, *P. australis* detritus and *H. tasmanica*

ω-Hydroxy acid	Percentage composition		
	P. australis (live fronds)	*P. australis* (detritus)	*H. tasmanica*
20:0	—	—	7.0
21:0	—	—	0.37
22:0	—	—	35.8
23:0	—	—	0.96
24:0	6.0	4.8	16.1
25:0	0.10	—	0.71
26:0	47.0	47.1	22.0
27:0	0.90	—	0.52
28:0	46.0	48.1	16.6
Total absolute concn (μg/g) dry wt	3450	240	150

ical studies by carefully choosing a variable, e.g. the absolute and relative abundance of a class of compounds or an individual component, or the components' chain-length distribution, may provide a useful chemical approach for distinguishing between seagrasses and other biological material as source components.

EXPERIMENTAL

The seagrasses *P. australis*, *H. tasmanica* and the sample of *P. australis* detritus were collected at Corner Inlet, Victoria. Prior to extraction of lipids, the seagrass samples were washed thoroughly in dist. H_2O to remove sediment and adhering detritus. The *P. australis* detritus sample, comprising decayed frond fragments (> 2.0 mm), was obtained by sieving a surface sediment sample. The material was not scraped to remove the encrusting material on the leaf surface.

Lipids were extracted from the samples using $CHCl_3$–MeOH (2:1) and separated into neutral and acid fractions [29, 30]. The fractions were further separated using chromatographic techniques previously described [18]. Bound lipids, obtained by saponification with MeOH–KOH (pH 12) of the solvent-extracted residues, were also fractionated using the same techniques used for the extractable lipids.

Analysis of neutral lipids. Sterol and alcohol TMSi ethers and hydrocarbon fractions were analysed by capillary GC on a glass SCOT SE30 column (45 m × 0.5 mm i.d., N_{eff} = 60000) temp. programmed from 150° to 280° at 2°/min. He was used as carrier gas (linear flow = 20 cm/sec). Sterol components were initially identified by co-chromatography with authentic standards where available and by comparison of RR_t values with published data [31].

Analysis of fatty acids. Me esters [32], after chromatographic fractionation, were analysed using the glass SE30 capillary column described above. Hydroxylated fatty acid Me esters were analysed as the corresponding TMSi ethers. Monocarboxylic Me esters were further analysed using a glass SP 1000 capillary column (100 m × 0.5 mm i.d., N_{eff} = 106 000) temp. programmed from 140° to 220° at 1.5° min. The polar SP 1000 capillary column was operated using He as carrier gas. Monocarboxylic acids were identified by co-chromatography with authentic standards where available and by RR_t [33, 34] and ECL measurements [35, 36]. All lipid components were quantificated by calibrated GC response and are subject to errors of ± 10%. Absolute concns (dry wt) are calculated using the mass of tissue remaining after solvent extraction and base saponification.

Sterols and ω-hydroxy acids were further identified by GC/MS using conditions previously described [37].

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