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THE STEROL AND FATTY ACID COMPOSITIONS OF SEVEN TROPICAL SEAGRASSES FROM NORTH QUEENSLAND, AUSTRALIA*

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Key Word Index—Seagrasses; chemotaxonomy; sterols; fatty acids.

Abstract—The sterol and fatty acid compositions of fresh leaves of the seagrasses *Cymodocea serrulata*, *Enhalus acoroides*, *Halodule uninervis*, *Halophila ovalis*, *H. ovata*, *H. spinulosa* and *Thalassia hemprichii* are reported. The major fatty acids were palmitic acid, linoleic acid and linolenic acid as expected. *H. ovalis* and *H. ovata* were characterized by the relatively high abundance (*ca* 5%) of the acid hexadeca-7,10,13-trienoic acid (16:3⟨7⟩). The sterol compositions were typical of higher plants, with sitosterol and stigmasterol accounting for 60–90% of the observed sterols. 28-Isofucosterol was a major sterol (20–30%) only in the *Halophila* spp. Cluster analysis of the sterol composition data clearly separated the *Halophila* spp. from the other seagrasses and enabled the distinction of *Enhalus* sp. from *Cymodocea*, *Halodule* and *Thalassia* spp. The seagrass species were clearly separated into five chemical groups using the combined fatty acid and sterol composition data and the need for a reappraisal of the taxonomic position of *Halophila* was indicated.

INTRODUCTION

Seagrasses are important marine primary producers in many tropical, sub-tropical and temperate regions of the world. They provide habitats for many species of fish and invertebrates, and form the base of many marine food chains. The analysis of gut contents has revealed that seagrasses are an important component of the diet for many commercial fish species [1, 2]. The threatened marine herbivorous mammal, the dugong (*Dugong dugon*), which ranges throughout tropical Australian waters, is totally dependent on seagrass meadows as its source of food [3]. In addition to this direct utilization, seagrass detritus and organic compounds released by decaying seagrasses may influence benthic microbial abundance and activity profoundly, and subsequently effect detritivore-based food webs.

Many studies on the occurrence of specific chemical compounds in seagrasses have been undertaken. In most cases, such studies have been directed towards problems of seagrass phylogeny. McMillan and co-workers [4, 5] examined the distributions of phenolic acids, sulphated phenolic compounds and flavone sulphates in seagrasses. They found marked family and generic differences in the occurrence of these compounds. Drew [6] proposed a seagrass phylogeny based upon the variations in the sugar and cyclitol compositions. Nichols *et al.* [7], as part of a programme directed towards the elucidation of carbon flow in food chains in temperate seagrass communities, proposed the use of lipids as distinctive chemical markers for the seagrasses *Posidonia australis* and *Heterozostera tasmanica*. A range of lipids was examined. The most abundant lipid classes were fatty acids, sterols and hydroxy fatty acids, with the latter being only minor constituents in *H. tasmanica*. Volkman *et al.* [8, 9] have performed detailed examinations of specific lipids in the seagrass *Zostera muelleri*, using high performance capillary gas chromatography and mass spectroscopy. Detailed analyses of other seagrasses using capillary GC/MS are lacking. Studies by Maurer and Parker [10], Attaway *et al.* [11], and McMillan [12] have reported packed column GC analyses of fatty acids and sterols in a small number of seagrasses. The sterol study [11] did not resolve the sterols 28-isofucosterol and sitosterol, and the fatty acid analyses did not include positional isomer identifications. Nevertheless, sterol compositions were shown to be chemotaxonomically significant [11], and leaf fatty acid compositions were shown to be only slightly affected by stresses such as chilling [12].

As a part of the Coastal Zone programme at the Australian Institute of Marine Science, it has been necessary to search for potential chemical markers which would enable the tracing of seagrass-derived material through the coastal ecosystems. In this study we report detailed fatty acid and sterol analyses of seven seagrass species collected from our study site at Magnetic Island, Queensland. Compositional variations between the different species were examined for potential chemotaxonomic significance by cluster analysis.

RESULTS AND DISCUSSION

Sterols

Table 1 lists the sterol compositions of the seven seagrass species examined in this study. In all cases only five sterols were detected and only three of these occurred

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Table 1. The sterol compositions of selected seagrasses

Sterol†	Species*							
	C.s.	*E.a.*	*H.u.*	*H.o.*	*H.ot.*	*H.s.*	*T.h.*	*T.h.*
27:1⟨5⟩	3.9	2.5	3.5	6.8	6.9	5.7	2.7	2.5
28:1⟨5⟩	4.3	5.9	6.7	5.8	7.4	3.6	6.9	5.8
29:2⟨5, 22E⟩	65.6	27.7	74.9	27.4	15.1	22.4	65.4	54.7
29:1⟨5⟩	22.8	62.8	14.4	30.8	46.9	43.6	22.4	32.3
29:2⟨5, 24(28)⟩	3.4	1.1	0.6	29.3	23.7	24.7	2.5	4.7

*C.s. = *Cymodocea serrulata* (R.Br.) Aschers & Magnus; *E.a.* = *Enhalus acoroides* (L.f.) Royle; *H.u.* = *Halodule uninervis* (Forsk.) Aschers; *H.o.* = *Halophila ovalis* (R.Br.) Hook., f.; *H.ot.* = *Halophila ovata* Gaud.; *H.s.* = *Halophila spinulosa* (R.Br.) Aschers; *T.h.* = *Thalassia hemprichii* (Enrenb.) Aschers (two samples were analysed).

†Sterol identifications: 27:1⟨5⟩ cholest-5-en-3β-ol (common name: cholesterol); 28:1⟨5⟩ 24-methylcholest-5-en-3β-ol (campesterol); 29:2⟨5,22*E*⟩ 24-ethylcholesta-5,22*E*-dien-3β-ol (stigmasterol); 29:1⟨5⟩ 24-ethylcholest-5-en-3β-ol (sitosterol); 29:2⟨5,24(28)⟩ 24-ethylcholesta-5,24(28)-dien-3β-ol (28-isofucosterol).

at greater than 20% abundance in any of the samples. The major sterols, sitosterol and stigmasterol, are typical abundant components in most higher plants, but 28-isofucosterol is not commonly very abundant [13]. Analyses of two samples of *Thalassia hemprichii* are reported. They were collected from different areas of the intertidal zone and differed markedly in their leaf pigmentation. We expected that these two samples might exhibit appreciable compositional differences. The *T. hemprichii* analyses are similar to the analyses of *Cymodocea serrulata* and *Halodule uninervis*, but are distinctly different to the analyses of the other seagrasses. If we assume that the compositional variation exhibited by the *Thalassia* samples is typical of seagrasses, then the seagrasses can only be divided into three chemical groups based solely on sterol compositions. *Halodule*, *Thalassia* and *Cymodocea* comprise one group (No. 1S) distinguished by a predominance of stigmasterol and sitosterol with a stigmasterol:sitosterol ratio of *ca* 2, *Enhalus* a second group (No. 2S) with stigmasterol and sitosterol dominant and a stigmasterol:sitosterol ratio of *ca* 0.5. *Halophila* constitutes a distinct group (No. 3S) characterized by the high abundance of 28-isofucosterol (20–30%). It is of interest to note that the reported analyses of the seagrasses *Posidonia australis* (Posidoniaceae) [7], *Heterozostera tasmanica* [7] and *Zostera muelleri* (Zosteraceae) [9], *Halophila engelmanni* and *T. testudinum* would place these species in the group with *Enhalus* (No. 2S). The chemical differentiation of the two *Thalassia* spp. is unexpected. The inclusion of a *Halophila* species in this group presumably results from the inability of the analytical techniques used in that study to resolve the sterols 28-isofucosterol and sitosterol. The two zannichelliacean species analysed by Attaway *et al.* [11] (*Diplanthera wrightii* and *Syringodium filiforme*) would both be included in group No. 1S whilst *Ruppia maritima* (Rupiaceae) [11] is chemically distinct from all three groups.

In order to obviate the differences in sterol compositions, cluster analysis was performed on the sterol data. The analytical data for each sterol were normalized to the mean and the separations of the data points in five-dimensional space were computed. Closest pairs were then clustered. Figure 1 is the resultant tree diagram. The three groupings proposed on the basis of visual inspection of the sterol analyses are clearly separated. The two *Thalassia* samples are well separated (Euclidean distance of approximately 0.6). If this is an accurate example of expected subsample variability, a Euclidean separation of approximately 1.0 is necessary before species can be considered to belong to distinct chemical groupings. Only the three groupings proposed are valid in the present case.

Fatty acids

Detailed fatty acid analyses of the seagrasses are reported in Table 2. Thirty fatty acids were identified. The three most abundant acids in all the species were linolenic acid (18:3⟨9⟩), linoleic acid (18:2⟨9⟩) and palmitic acid (16:0). These acids accounted for more than 80% of the total fatty acids observed. In addition to these major acids, six other fatty acids occurred at relative abundances of greater than 2% in at least one species, viz. hexadec-9-enoic acid (16:1⟨9⟩, common name: palmitoleic acid), *trans*-hexadec-3-enoic acid (16:1⟨3*t*⟩), hexadeca-7,10,13-trienoic acid (16:3⟨7⟩), octadecanoic acid (18:0, stearic acid), octadec-9-enoic acid (18:1⟨9⟩, oleic acid), and

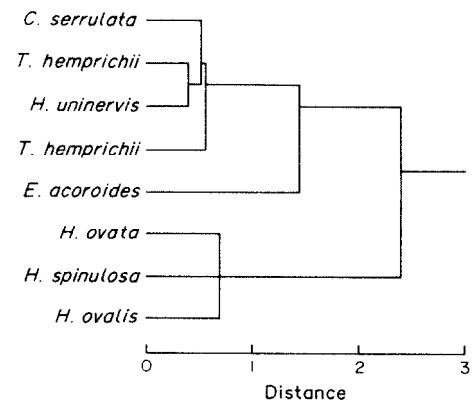

Fig. 1. Tree diagram derived by cluster analysis of the sterol composition data. Note: *H. uninervis* is *Halodule uninervis* whilst the other '*H.*' species refer to the genus *Halophila*.

Table 2. The fatty acid compositions (%) of North Queensland seagrasses

	Species*							
Fatty acid	*C.s.*	*E.a.*	*H.u.*	*H.o.*	*H.ot.*	*H.s.*	*T.h.*	*T.h.*
12:0	0.07	0.13	0.22	0.10	0.16	0.47	0.21	0.16
14:1⟨7⟩	0.05	0.43	0.37	0.17	0.03	0.21	0.11	0.08
14:0	0.35	0.43	0.59	0.59	0.99	0.68	0.64	0.59
15:0	0.36	0.20	0.29	0.29	0.23	0.33	0.21	0.20
16:3⟨7⟩	0.07	0.30	0.04	4.64	5.04	0.39	0.35	0.33
16:2⟨7⟩	0.04	0.18	0.03	0.81	0.60	0.11	0.06	0.04
16:2⟨9⟩	0.09	0.79	1.58	0.11	0.10	1.08	0.83	0.52
16:1⟨7⟩	0.27	0.39	0.50	0.79	0.62	0.70	0.43	0.36
16:1⟨9⟩	2.53	0.38	2.89	0.43	1.24	0.38	0.49	0.43
16:1⟨3*t*⟩	2.33	2.07	2.57	1.64	2.46	1.76	1.70	1.59
16:0	19.2	26.7	25.0	20.4	22.5	35.3	24.2	25.8
17:1⟨9⟩	0.38	0.21	0.32	1.57	0.49	0.21	0.16	0.11
17:0	0.46	0.24	0.29	0.20	0.18	0.53	0.23	0.25
18:3⟨9⟩	48.6	46.2	35.5	55.0	46.3	24.3	47.0	44.6
18:2⟨9⟩	18.5	12.8	19.4	8.19	12.4	11.3	16.9	18.2
18:1⟨9⟩	3.33	1.21	3.22	2.40	2.98	2.55	1.78	1.90
18:1⟨11⟩	0.55	0.32	0.39	0.59	0.68	0.60	0.44	0.38
18:0	0.98	3.91	3.04	1.71	2.05	9.94	2.70	3.38
20:3⟨9⟩	0.03	0.21	0.07	0.00	0.29	0.04	0.13	0.10
20:2⟨9⟩	0.09	0.19	0.23	0.00	0.05	0.15	0.14	0.14
20:1⟨9⟩	0.10	0.12	0.32	0.03	0.08	0.13	0.13	0.16
20:0	0.13	0.42	0.43	0.12	0.20	1.71	0.33	0.32
21:0	0.07	0.20	0.19	0.04	0.00	0.74	0.07	0.05
22:0	0.32	0.28	0.27	0.15	0.20	2.50	0.32	0.24
23:0	0.27	0.10	0.14	0.06	0.04	1.41	0.07	0.04
24:0	0.28	0.07	0.26	0.08	0.11	1.37	0.11	0.06
25:0	0.06	0.01	0.06	0.01	0.00	0.29	0.02	0.00
26:0	0.03	0.01	0.03	0.01	0.01	0.44	0.02	0.00
27:0	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00
28:0	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00

*See Table 1.

docosanoic acid (22:0). All other acids were relatively minor in abundance.

The two *Thalassia* samples were very similar in composition: the maximum abundance difference was only 2.4%, which was for the acid 18:3⟨9⟩, and the maximum fractional variation in percentage abundance (considering only the nine 'major' acids) occurred with 18:0 (difference = 0.68%, mean = 3.04%). The variation observed between the species was much greater. For example, the abundance of 18:3⟨9⟩ varied in the range 24–55%, and 18:0 varied from 1.0 to 9.9%. With interspecific variation so much greater than the subsample variation, it should be possible to distinguish many of the species on the basis of their fatty acids.

The analysis of *H. spinulosa* reported (Table 2) is clearly different to all the other species; the saturated fatty acids (16:0, 18:0, 20:0, 21:0, 22:0, etc.) are markedly more abundant than in the other samples, and 18:3⟨9⟩ is appreciably less abundant. The fatty acid composition of *H. spinulosa* is not at all similar to the compositions of the other two *Halophila* species analysed. Both *H. ovalis* and *H. ovata* contain *ca* 5% of 16:3⟨7⟩ which distinguishes them from the other seagrasses. This acid has been reported to constitute 3–5% of the fatty acids of the seagrasses *Zostera muelleri* [8] and *Heterozostera tasmanica* [7], but is absent from *Posidonia australis* [7]. Pending further analyses, it is suggested that this acid is characteristic of the Zosteraceae and *Halophila* spp. Although *H. spinulosa* is currently placed in a different section of the genus from the other two species analysed, the anomalous composition of *H. spinulosa* still needs further explanation. *Cymodocea serrulata* and *Halodule uninervis* are distinguishable from the other seagrass species by the greater abundance of the acid 16:1⟨9⟩ (2.5–3% cf. 0.3–1.2%).

Cluster analysis was performed on the normalized fatty acid data (using only the nine major acids), resulting in the tree diagram (Fig. 2). Four groupings are evident, viz. *Cymodocea* and *Halodule* (No. 1F), *Thalassia* and *Enhalus* (No. 2F), *H. ovalis* and *H. ovata* (No. 3F), and finally the atypical species *H. spinulosa* (No. 4F). Since the sterol cluster analysis readily distinguished *Enhalus* and *Thalassia*, it is evident that combined application of sterol and fatty acid analyses can divide the seagrasses into at least five chemical groups. Indeed, the reported analyses of *Heterozostera* sp. [7] and *Zostera* sp. [8, 9] would result in these two genera being grouped in a sixth subdivision.

Chemical markers

In order to trace chemically the transport of material derived from seagrass from its origin in a seagrass bed to

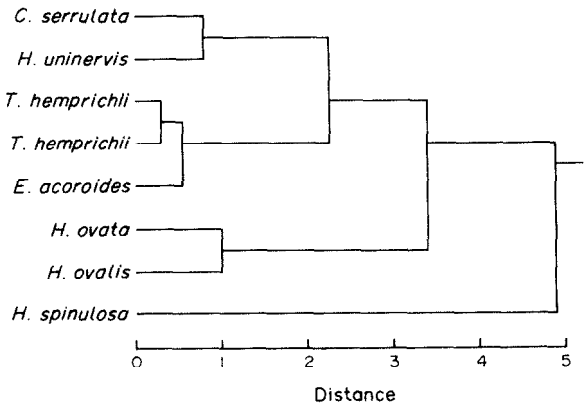

Fig. 2. Tree diagram derived by cluster analysis of the fatty acid distribution (using only those acids which occurred at abundances of > 2% in at least one sample, i.e. 16:3⟨7⟩, 16:1⟨9⟩, 16:1⟨3t⟩, 16:0, 18:3⟨9⟩, 18:2⟨9⟩, 18:1⟨9⟩, 18:0 and 22:0).

neighbouring ecosystems, it is necessary to have specific markers that are both characteristic of the seagrass species (or group of species) and refractory. Unsaturated fatty acids do not survive even short exposures to oxic detrital environments [7, 14] and are thus unsuitable for this type of study. In contrast, sterols have been reported to occur in sediments greater than 20 000 years old [15]. Indeed, in several recent studies, Johns and co-workers [9, 16] have proposed that some of the sterols found in subsurface intertidal sediments were derived from *Zostera muelleri* detritus. However, Nichols *et al.* [7] demonstrated that quantitative transfer of the sterols to sediments does not occur in the case of *Posidonia australis*, which directs a cautionary approach to relict sterols in sediments.

In the present study we have shown that seagrasses can be divided into at least three chemical groups on the basis of their sterol compositions. Thus, in environments where several species exist in close proximity (as found in North Queensland), it is theoretically possible to trace different subsets of the seagrass community into herbivore food chains. In addition, if the efficiency of transmission of sterols to the underlying sediments is similar for the various seagrass species and turbation is low, it should be possible to use sediment depth profiles as temporal records of past seagrass populations at the study site.

Chemotaxonomy

Combined cluster analysis using sterol plus fatty acid analyses establishes three major groupings amongst the seven species studied. Two of these are in excellent agreement with current seagrass taxonomy, namely *Cymodocea* plus *Halodule*, both in the Zannichelliaceae, and *Thalassia* plus *Enhalus*, both in the Hydrocharitaceae. However, the wide separation of the three *Halophila* species from all the others belies their present inclusion together with *Thalassia* and *Enhalus* in the Hydrocharitaceae. *Halophila* is separated from the other seagrasses analysed at a much higher level than either of the other species in that family, and also considerably higher than the level of separation between the two families. Data presented by Drew [6] for cyclitol composition also established clear chemical differences between these two families but because the hydrocharitacean species, in contrast to the zannichelliacean species, lacked any complexity of cyclitol composition, no differentiation within that family was possible. It has long concerned one of us (E.A.D.) that the leaves of *Halophila* are so radically different from all other seagrasses, and are in fact strikingly similar to another family of aquatic plants, the Potamogetonaceae. Although this alone would be insufficient to suggest major taxonomic separation, in conjunction with the sterol and fatty acid data there is now a sound basis for a reappraisal of the taxonomic position of *Halophila* in future chemical taxonomic studies.

EXPERIMENTAL

The seagrasses *C. serrulata*, *E. acoroides*, *H. uninervis*, *H. ovalis*, *H. ovata*, *H. spinulosa* and *T. hemprichii* were collected at Magnetic Island, Queensland. The samples were washed free of detritus and sediment with distilled H_2O, and then extracted with $CHCl_3$–MeOH (1:1) as described elsewhere [13]. Sterols and fatty acid methyl esters were prepared from the extracts by standard procedures [13, 17, 18].

Gas chromatographic analyses. Sterols (as the methyl ethers [13]) and fatty acid methyl esters were analysed by GLC using a fused silica SP2100 column (50 m × 0.2 mm i.d., Hewlett–Packard) as described previously [13, 18], and fatty acid esters were additionally analysed on a polar Superox 0.1 fused silica capillary column (50 m × 0.2 mm i.d., S.G.E. Australia). GC/MS analyses, to confirm sterol structures, were performed on a fused silica cross-linked methyl silicone column (50 m × 0.2 mm i.d., 0.11 μm phase thickness, ULTRA #1, Hewlett–Packard) with the HP5970A mass selective detector directly coupled to the gas chromatograph. Ionization voltage was 70 eV. Spectra were scanned repetitively at 600 amu/sec.

Fatty acids and sterols were identified by their mass spectra and by co-chromatography with standards where available. Authentic standards of sterols were courtesy of the Medical Research Council (U.K.).

All data analysis was performed on a PDP-11/70 minicomputer using the BMDP statistics package.

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