Phytochemistry, Vol. 24, No. 1, pp 81–84, 1985
Printed in Great Britain

0031–9422/85 $3.00 + 0.00

LIPIDS OF THE TROPICAL SEAGRASS *THALLASSIA HEMPRICHII**

PETER D. NICHOLS† and R. B. JOHNS‡

Department of Organic Chemistry, University of Melbourne, Parkville, Victoria, Australia 3052

(*Received* 5 *April* 1984)

Key Word Index—*Thallassia hemprichii*; Hydrocharitaceae; seagrass; lipids; hydrocarbons; sterols; monocarboxylic acids; α,ω-dicarboxylic acids; ω-hydroxy acids; α-hydroxy acids; chemical marker; food chain.

Abstract—The component sterols, alcohols, hydrocarbons, monocarboxylic, α,ω-dicarboxylic and α- and ω-hydroxy acids from the leaves and roots of the tropical seagrass *Thallassia hemprichii* are reported. The leaves contained significant concentrations of cholest-5-en-3β-ol, a sterol not normally detected in either higher plants or seagrasses. The lower abundance of polyunsaturated fatty acids found in both the leaves and roots compared to other seagrass species may be a result of the warmer waters from which this species was collected. Solvent-extractable, long-chain ($> C_{22}$) α,ω-diacids, α- and ω-hydroxy and monocarboxylic acids were also isolated from the leaves. The distribution pattern of these lipids should enable these components along with other distinctive components to be used as chemical markers for this seagrass.

INTRODUCTION

Seagrasses are important primary producers in the marine environment and provide food, shelter and nursery grounds for many crustaceans and fish fauna [1]. An interdisciplinary study of the temperate beds of seagrasses at Corner Inlet, Victoria has been examining food-chain structure and geochemical characteristics of the seagrass communities. A number of techniques have been utilized in the course of the project and include biochemical analysis of the digestion of seagrasses, microscopic and macroscopic examination of animal gut contents [2–4], and the analysis of various lipid classes present in the seagrasses [5, 6] and associated organisms [6, 7] for use as chemical markers. In a report on the lipid composition of the seagrasses *Posidonia australis* and *Heterozostera tasmanica*, several lipid components were suggested as potential markers for monitoring seagrass contribution to both food webs and marine sediments [5].

In this report the lipid components of the leaves and roots of the tropical seagrass *Thallassia hemprichii* collected from Lizard Island, North Queensland have been analysed for comparative purposes. The lipid classes investigated included sterols, alcohols, hydrocarbons, monocarboxylic, α,ω-dicarboxylic and α- and ω-hydroxy fatty acids. The data obtained for *T. hemprichii* represent a small part of an ongoing project aimed at (i) providing base-line lipid composition data of selected Great Barrier Reef marine organisms and (ii) utilizing such lipid data for assessing existing inter-relationships in the food web.

RESULTS AND DISCUSSION

The sterol composition of the leaves of *T. hemprichii* (Table 1) differs from that reported for other seagrasses [5, 8], including *T. testadium* [8]. Cholest-5-en-3β-ol, a sterol not normally detected at high concentration in higher plants, is a major sterol detected in *T. hemprichii* (25% of total sterols, 90 ppm). The detection of cholest-5-en-3β-ol, together with a range of other sterols not commonly found in higher plant material, in a sample of *P. australis* detritus was shown to be due to the presence of associated epiphytic material [5]. If the cholest-5-en-3β-ol found in *T. hemprichii* was due to the presence of some other form of epiphytic biological material, elevated levels of polyunsaturated fatty acids (PUFAs) not present in seagrass lipids but common in many marine organisms (e.g. 20:4ω6§ and 20:5ω3) would be detected, as was observed for the sample of *P. australis* detritus. These acids were detected at only a very low concentration in *T. hemprichii*, which for this study had been rigorously washed to remove adhering material. The high proportion of cholest-5-en-3β-ol detected in duplicate analyses of *T. hemprichii* is thus a chemotaxonomic feature of this seagrass.

Other sterols detected in the leaves of *T. hemprichii* were 24-methylcholest-5-en-3β-ol, 24-ethylcholesta-5,22*E*-dien-3β-ol, 24-ethylcholest-5-en-3β-ol, 24-ethyl-5α-cholestan-3β-ol and 24-ethylcholesta-5,24(28)*Z*-dien-3β-ol (isofucosterol). The latter two components co-elute on the capillary columns used in this study and their relative abundances were determined by GC/MS analysis. Isofucosterol has previously been reported in Liliaceae [9], mangrove leaves [10] and other terrestrial plants, and its presence in *T. hemprichii* and *Zostera meulleri* [11] further widens the range of biological samples within which this sterol occurs. 24-Ethylcholest-5-en-3β-ol is generally the major sterol detected in higher plant leaves; however, in the leaves of *T. hemprichii* 24-ethylcholesta-5,22*E*-dien-3β-ol was the major sterol isolated.

The major sterols found in the roots of *T. hemprichii*—

*Part VIII in the series "A Study of Food Chains in Seagrass Communities". For Part VII see *Comp. Biochem. Physiol.* (in press).

†Present address: Department of Biological Science, The Florida State University, Tallahassee, FL 32306, U.S.A.

‡To whom reprint requests should be addressed.

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

Table 1. Sterol composition of *Thallassia hemprichii*

RR_t*	Sterol	Percentage composition of total sterols: Leaves	Roots
1.00	Cholest-5-en-3β-ol	25.0	1 0
1.12	24-Methylcholesta-5,22*E*-dien-3β-ol	—	0.5
1.31	24-Methylcholest-5-en-3β-ol	6.2	19.5
1.42	24-Ethylcholesta-5,22*E*-dien-3β-ol	31.7	26.3
1.63	24-Ethylcholest-5-en-3β-ol	30.1	52.7
1.68	24-Ethyl-5α-cholestan-3β-ol	3 5	—
1.68	24-Ethylcholesta-5,24(28)*Z*-dien-3β-ol	3.4	—
	Total concentration, μg/g (dry wt)	370	770

*SE30 fused silica column, RR_t cholest-5-en-3β-ol = 1.00; 24-ethylcholest-5-en-3β-ol = 1.63.

24-methylcholest-5-en-3β-ol, 24-ethylcholesta-5,22*E*-dien-3β-ol and 24-ethylcholest-5-en-3β-ol—are all commonly found in higher plants. Cholest-5-en-3β-ol is again present; however, its relative abundance in marked contrast to that found in the leaves represents only 1% of the total sterols.

A series of straight-chain alcohols were identified in the neutral lipids of both plant segments analysed (Table 2). Long-chain alcohols, maximizing at n-C_{26} in the leaves and n-C_{24} in the roots, were detected in both samples, and represented 20.7 and 91.1%, respectively, of the total alcohols present. The predominance of even chain-saturated components and the absence of branching or unsaturation are typical of higher plant leaf waxes [9]. Phytol, the side chain of the chlorophyll molecule, was, as expected, the major alcohol component in the leaf sample.

Table 2. Acyclic alcohol composition of *Thallassia hemprichii*

Alcohol	Percentage composition: Leaves	Roots
12:0	—	tr
14:0	0.2	0.4
16:0	5.5	1.1
17:0	—	tr
18:0	3.0	2.0
Phytol	60.5	5.7
20:0	1.2	3.0
21:0	—	1.6
22:0	3.8	10.5
23:0	tr	2.1
24:0	4.4	22.6
25:0	tr	1.1
26:0	5.6	21.8
27:0	1.9	7.8
28:0	2.2	12.7
29:0	—	tr
30:0	1.6	5.1
32:0	—	2.8
Total concentration (μg/g dry wt)	3150	260
$\geq C_{20}$ (μg/g dry wt)	650	240
Total lipid (%, dry wt basis)	5.0	1.0

tr = trace, < 0.1%.

Hydrocarbon profiles similar to those occurring in the temperate seagrass species collected at Corner Inlet, *P. australis* and *H. tasmanica* (i.e. n-alkanes maximizing at n-C_{17} or n-C_{19} with a low abundance of long-chain components [5]), were observed for both segments of *T. hemprichii*. Two homologous unidentified cyclic alcohols were also detected in the saponified neutral lipid fraction isolated from the root sample.

Monocarboxylic acids represented the major lipid class detected in both the leaves and roots of *T. hemprichii* (Table 3) and the major acids in decreasing order of abundance were 16:0, 18:3ω3, 18:2ω6, 18:1ω9 (18:1ω7 was the major C_{18} monounsaturated acid detected in the root sample), 16:1ω7, 24:0 and 22:0. These acids combined totalled 82 and 87% of the monoacids isolated from the leaves and roots, respectively. The C_{16} PUFAs 16:2ω6 and 16:3ω3, previously reported in the lipids of *Z. meulleri* [11] and *Heterozostera tasmanica* [5], were not detected in *T. hemprichii*. The C_{18} PUFAs 18:2ω6 and 18:3ω3 were also present at lower relative concentrations than for the other seagrasses. This trend, apart from indicating differences between the seagrass species, may be due to the differing environment of location for *T. hemprichii*, which was collected from the tropical waters of Lizard Island. The inverse relationship between temperature and degree of unsaturation is well documented (e.g. [12, 13]) and the data presented here for seagrass lipids may well be another example of this trend.

Long-chain ($\geq C_{20}$) saturated monoacids were only minor components (Table 3) of the total monoacids in both samples and maximized at 24:0 in both the leaves and roots. The low concentration of long-chain monoacids coupled with the absence of the α,ω-diacid and ω-hydroxy acid components commonly found in great abundance in higher plant leaves (i.e. 16:0, 18:0 and 18:1) is, as was noted for *P. australis* and *H. tasmanica*, probably due to poor cuticle development in seagrasses. This fact supports the theory that plants submerged in seawater do not require the same structural development as is noted for the leaves of terrestrial plants [14].

Two long-chain α,ω-dicarboxylic acids, di-28:0 and di-30:0, and their analogous ω-hydroxy acids were detected in both the leaves and roots of *T. hemprichii* (Tables 3 and 4). The nature of the distribution pattern of both α,ω-diacids and ω-hydroxy acids in seagrasses has been discussed previously [5] and analysis of the data pre-

Table 3. Mono- and α,ω-dicarboxylic acid composition of *Thallassia hemprichii*

Acid	Percentage composition Leaves	Roots
12:0	tr	—
14:0	0.3	0.4
i 15:0	0.1	tr
a 15:0	0.1	—
15:0	0.5	0.6
16:1ω7	1.7	1.8
16:1ω5	0.3	tr
16:0	25.0	30.8
i 17:0	tr	—
17:0	0.5	0.5
18:3ω3 } 18:2ω6 }	39.4	35.0
18:1ω9	10.1	4.6
18:1ω7	0.2	9.5
18:0	2.9	3.5
19:0	0.1	tr
20:4ω6	0.3	—
20:5ω3	0.4	—
20:1ω9	0.4	tr
20:0	0.6	tr
21:0	0.2	tr
22:4ω6	0.2	—
22:5ω3	0.2	—
22:0	1.0	1.0
23:0	0.6	0.5
24:0	1.5	1.2
25:0	0.3	0.3
26:0	0.5	0.4
27:0	0.1	tr
28:0	0.6	0.4
29:0	tr	—
30:0	0.4	tr
Di-28:0	1.6	1.0
Di-30:0	3.3	2.0
Unidentified	7.0	5.9
Total concentration (μg/g dry wt)		
(i) Monoacids	5020	1090
(ii) α,ω-Diacids	260	30

tr = trace, < 1%.

Table 4. α- and ω-hydroxy acid composition of *Thallassia hemprichii*

Hydroxy acid	Percentage composition Leaves	Roots
ω-OH 26:0	4.4	—
ω-OH 28:0	47.5	54.5
ω-OH 30:0	48.1	45.5
Total composition (μg/g dry wt)	90	30
α-OH 22:0	19.7	—
α-OH 23:0	8.1	—
α-OH 24:0	57.3	tr
α-OH 25:0	5.8	—
α-OH 26:0	9 1	—
Total composition (μg/g dry wt)	15	n.d.

n.d. = not determined. tr = trace, < 0.5%.

sented here for *T. hemprichii* again indicates the differing role of these components in seagrass compared with terrestrial higher plants. The distribution patterns of long-chain (C_{28}, C_{30}) mono-, α,ω-di- and ω-hydroxy acids in the leaves and roots of *T. hemprichii* are consistent with the view that oxidative conversion of monocarboxylic acids to ω-hydroxy acids followed by conversion of ω-hydroxy acids to α,ω-diacids is occurring in this seagrass. These interconversions have been reported previously in higher plants [15].

A series of α-hydroxy acids, ranging from 22:0 to 26:0, even carbon predominating and maximizing at 24:0, were detected only in the leaves of *T. hemprichii* (Table 4). α-Hydroxy fatty acids have been reported previously in the leaves of *Z. meulleri* and in associated sediments [11].

The data presented in this report provide natural levels of a range of lipid classes detected in the tropical seagrass *Thallassia hemprichii*. The distinctive distribution pattern and range of α,ω-diacids and α- and ω-hydroxy acids coupled with the relative and/or absolute abundance of other components should allow the input of this seagrass into either tropical sediments or food webs to be monitored.

EXPERIMENTAL

Samples of *T. hemprichii* (Ehrenbs) Aschers were collected at Lizard Island, North Queensland and immediately stored at 0°. Prior to extraction of lipids, the seagrass samples were washed thoroughly with distilled H_2O to remove sediment and adhering detritus. Lipids were extracted using $CHCl_3$–MeOH (2:1) with 0.5% pyridine followed by alkaline saponification of the total extract. The total neutrals were extracted exhaustively from the alkaline aq. soln using heptane–$CHCl_3$ (4:1) and on acidification of the aq. layer, the total acids were recovered by solvent extraction in the same solvent mixture [16, 17]. The fractions were further separated using chromatographic techniques previously described [6, 18] or analysed directly by GC.

Analysis of neutral lipids. Sterol and alcohol TMSi ethers and hydrocarbon fractions were analysed by capillary GC on a fused silica (25 m × 0.2 mm i.d.) SE 30 column, temp. programmed from 100° to 300° at 3°/min after injection of the sample at 50°. H_2 was used as carrier gas with N_2 as make-up gas. Sterol components were initially identified by co-chromatography with authentic standards and previously identified sterols and by comparison of RR_t values with published data [19, 20].

Analysis of fatty acids. Me esters [21] were analysed using the fused silica SE 30 capillary column described above. Hydroxylated fatty acid Me esters were analysed as the corresponding TMSi ethers. Monocarboxylic acids were identified by co-chromatography with authentic standards where available and by ECL measurements. All lipid components were quantified by calibrated GC response and are subject to errors of up to 10%. Absolute concns (dry wt) were calculated using the mass of tissue remaining after solvent extraction.

GC/MS analyses. Analysis of all lipid samples was performed on a system fitted with an open split interface between the capillary (SE 30 fused silica, 50 m × 0.2 mm i.d.) column and the spectrometer. Samples were injected (splitless mode) at 50°, after which the oven was programmed from 140° to 300° at 4°/min; He was used as carrier gas (0 7 ml/min). The transfer line, source and

analyser were maintained at 280°. General operating parameters (peak finder mode) included: autotune file DFTP normalized; optics tuned at m/z 502; MS peak detect threshold = 10.0 linear counts; 4 samples per 0.1 amu; GC peak detect threshold = 150 triggered on total ion abundance; electron impact energy = 70 eV.

Acknowledgements—Mr. R. J. Western, CSIRO Division of Materials Science, is thanked for access to the GC/MS system used in this study. The Lizard Island research station staff are acknowledged for their consideration and assistance during the 1982 field trip. P.D.N. acknowledges financial assistance from a University of Melbourne postgraduate award. Dr. I. Poiner, CSIRO, Marine Laboratories, Cleveland is thanked for seagrass identification.

REFERENCES

1. Phillips, R. C. (1978) *Oceanus* **21**, 30.
2. Klumpp, D. W. and Nichols, P. D. (1983) *Aust. J. Mar. Fresh Water Res.* **34**, 745.
3. Klumpp, D. W. and Nichols, P. D. (1983) *Mar. Ecol. Prog. Ser.* **12**, 207.
4. Klumpp, D. W. and Nichols, P. D. (1983) *Mar. Biol. Letters* **4**, 331.
5. Nichols, P. D., Klumpp, D. W. and Johns, R. B. (1982) *Phytochemistry* **21**, 1613.
6. Nichols, P. D. (1983) Ph.D. Thesis, University of Melbourne.
7. Nichols, P. D., Klumpp, D. W. and Johns, R. B., *Comp. Biochem. Physiol.* (in press).
8. Attaway, D. H., Haug, P. and Parker, P. L. (1971) *Lipids* **6**, 687.
9. Kolattakudy, P. E. (1970) *Lipids* **5**, 259.
10. Wannigama, G. P., Volkman, J. K., Gillan, F. J., Nichols, P. D. and Johns, R. B. (1981) *Phytochemistry* **20**, 659.
11. Volkman, J. K., Gillan, F. T., Johns, R. B. and Eglinton, G. (1981) *Geochim. Cosmochim. Acta* **45**, 1817.
12. Gill, C. O. and Suisted, J. R. (1978) *J. Gen. Microbiol.* **104**, 31.
13. Mumma, R. O., Fergus, C. L and Sekura, R. D. (1970) *Lipids* **5**, 100
14. Nevenzel, J. C. (1970) *Lipids* **5**, 308.
15. Kolattukudy, P. E., Croteau, R. and Buchner, J. S. (1976) in *Chemistry and Biochemistry of Natural Waxes* (Kolattukudy, P. E., ed.), pp. 289–347. Elsevier, Amsterdam.
16. Gillan, F. T., McFadden, G., Wetherby, R. and Johns, R. B (1981) *Phytochemistry* **20**, 1935.
17. Johns, R. B, Nichols, P. D. and Perry, G. J. (1980) *Comp. Biochem. Physiol.* **65B**, 207.
18. Perry, G. J, Volkman, J. K., Johns, R. B. and Bavor, H. J. (1979) *Geochim. Cosmochim. Acta* **43**, 1715.
19. Patterson, G. W. (1971) *Analyt. Chem.* **43**, 1165.
20. Itoh, T., Tani, H., Fukushima, K., Tamura, T. and Matsumoto, T. (1982) *J. Chromatogr.* **234**, 65
21. Metcalf, L. D. and Schmitz, A. A. (1961) *Analyt. Chem.* **33**, 363.