13. Carter, J. and Smith, E. E. (1973) *Arch. Biochem. Biophys.* **155**, 82.
14. Takahashi, N. and Shimomura, T. (1973) *Agric. Biol. Chem.* **37**,67.
15. Jorgensen, B. B. and Jorgensen, O. B. (1967) *Biochim. Biophys. Acta* **146**, 167.
16. Galand, G. and Forstner, G. G. (1974) *Biochem. J.* **144**, 281.
17. Semenza, G., Curtius, H.-Ch., Raundhardt, O., Hore, P. and Muller, M. (1969) *Carbohydrate Res.* **10**, 417.
18. Reese, E. T., Maguire, A. H. and Parrish, F. W. (1968) *Can. J. Biochem.* **46**, 25.
19. Abdullah, M., Fischer, E. H., Querishi, M. Y., Slessor, K. N. and Whelan, W. J. (1965) *Biochem. J.* **97**, 9P.
20. Jones, G. and Whelan, W. J. (1969) *Carbohydrate Res.* **9**, 483.
21. Abdullah, M. and Whelan, W. J. (1960) *Biochem. J.* **75**, 12P.
22. Manners, D. J. (1974) in *Plant Carbohydrate Biochemistry* (Pridham, J. B., ed.) p. 109. Academic Press. London.
23. Aspinall, G. O. and Ferrier, R. J. (1957) *J. Chem. Soc.* 4191.
24. Trevelyan, W. G., Procter, D. P. and Harrison, J. S. (1950) *Nature* **166**, 444.
25. Ornstein, L. (1964) *Ann. N.Y. Acad. Sci.* **121**, 321.
26. Vesterberg, O. and Svensson, H. (1966) *Acta Chem. Scand.* **20**, 820
27. Andrews, P. (1964) *Biochem. J.* **91**, 222.
28. O'Carra, P. (1965) *Biochem. J.* **94**, 171.

Phytochemistry, 1978, Vol 17, pp 1431–1432 © Pergamon Press Ltd. Printed in England 0031-9422/78/0801-1431 $02 00/0

HYDROCARBONS OF *MACROCYSTIS PYRIFERA* BLADES

STEVEN S. ROSSI, GEORGE W. ROMMEL and ANDREW A. BENSON
Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla, CA 92093, U.S.A.

(*Revised received* 18 *January* 1978)

Key Word Index—*Macrocystis pyrifera*; Lessoniaceae; giant kelp; hydrocarbons.

INTRODUCTION

Among lipids in benthic marine algae, hydrocarbons are least understood and they are known only from analyses of plants common to the Atlantic seaboard [1]. Hydrocarbons in macroalgae of Pacific Ocean waters are even less well characterized. We wish to report the characterization of the major saturated and unsaturated hydrocarbons in the giant kelp (*Macrocystis pyrifera*), a prominent brown alga of west coastal waters.

RESULTS AND DISCUSSION

Hydrocarbon constituents of *Macrocystic pyrifera* blades are given in Table 1. Dry material comprised 7 ± 1.8% (s.d.) of the fr. wt, and 4.4 ± 1.3 mg (0.1 ± 0.03% dry wt) non-saponifiable lipid material was extracted. Total hydrocarbons accounted for between 0.4 and 0.6% of these lipids. Saturated hydrocarbons made up from 43 to 48% of the total and *n*-alkanes were the dominant saturated species (62.4% of total saturates). Branched alkanes were generally absent from giant kelp blades.

Together, the alkenes 3,6,9,12,15,18-heneicosahexaene (HEH) and 3,6,9,12,15-heneicosapentaene (HEP) accounted for 51.5–81% of the total unsaturates present. It is presumed that HEH and HEP contain all-*cis* saturated bonds, as observed in blades of the closely related alga, *Laminaria saccharina* [1]. Squalene (2,6,10,-15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene), which has not been reported in earlier work with marine algae [1], was an important constituent in giant kelp blades, comprising 5.1–19.2% of the total hydrocarbon.

The amounts of *n*-alkanes in *M. pyrifera* blades are presented in Table 2. The dominant alkane was *n*-pentadecane, which comprised from 67.9 to 92.2% of the total *n*-alkanes, accounting for roughly 15% of the total hydrocarbons in kelp blades. Lesser, yet significant, concentrations of *n*-heptadecane were observed. Alkanes within the range C_{18}–C_{22} were generally absent. Saturated molecules with chain lengths greater than C-32 were also poorly represented. Odd-number carbon chain length compounds occurred in greater abundance than did even-number alkanes, resulting in high carbon preference indices. All the above characteristics are common to other brown algae species [2, 3]. Samples collected from two locations, San Miguel Island and Coal Oil Point, exhibited *n*-alkane profiles which deviated from the above, and suggest the presence of petroleum contamination. Total hydrocarbon levels in these samples were also elevated when compared to others. Several characteristic components of petroleum were also present in suspect samples, and absent from others viz. (a) a mixture of unresolved complex (branched and cyclic) hydrocarbons (U.C.M.), particularly pronounced in saturate fractions; (b) significant concentrations of *n*-alkanes in the range C_{18}–C_{22}; simultaneous occurrence of pristane and phytane (trace quantities detected only in suspect kelp samples); (c) reduced predominance of odd-number chain length *n*-alkanes C_{23}–C_{30} [4]. Retention indices of polynuclear aromatic hydrocarbon standards did not correlate with those of any compounds presently noted in *M. pyrifera* blades. Aromatic hydrocarbons have been detected in severely polluted marine Chlorophyceae, but were absent from algae sampled from pristine locations [4]. One must stress

Table 1. Hydrocarbon composition of *Macrocystis pyrifera* blades

Hydrocarbons	Unpolluted (6)	Polluted (11)
Saturated		
n-alkanes	1.39 ± 0.37	4.71 ± 0.21
branched	ND	0.21 ± 0.08
unresolved	0.82 ± 0.14	3.45 ± 0.69
Total	2.21 ± 0.23	8.37 ± 0.95
Unsaturated		
HEH and HEP	1.86 ± 0.22	2.19 ± 0.90
squalene	0.51 ± 0.35	0.62 ± 0.31
branched	0.31 ± 0.25	0.39 ± 0.16
unresolved	ND	1.99 ± 0.74
Total	2.68 ± 0.30	5.19 ± 0.56
Total hydrocarbons	4.89 ± 0.28	13.56 ± 0.74

All values are mean ± s.d. μg/g dry wt; number of samples given in parentheses. Abbreviations: HEH = heneicosahexaene; HEP = heneicosapentaene; ND = not detected. Unpolluted samples collected from two offshore islands (San Clemente and San Nicolas) and Corona del Mar (coastal); polluted samples were taken from an offshore island (San Miguel) and Coal Oil Point (coastal).

Table 2. *n*-Alkanes of *Macrocystis pyrifera* blades

n-Alkane	Unpolluted	Polluted
C_{14}	ND	ND
C_{15}	1.16 ± 0.05	1.51 ± 0.42
C_{16}	ND	ND
C_{17}	0.06 ± 0.02	0.05 ± 0.03
C_{18}	ND	0.01 ± 0.01
C_{19}	ND	0.02 ± 0.02
C_{20}	ND	0.07 ± 0.04
C_{21}	t	0.04 ± 0.02
C_{22}	t	0.18 ± 0.07
C_{23}	t	0.20 ± 0.07
C_{24}	t	0.48 ± 0.16
C_{25}	0.03 ± 0.02	0.54 ± 0.18
C_{26}	0.03 ± 0.01	0.51 ± 0.13
C_{27}	0.04 ± 0.02	0.27 ± 0.09
C_{28}	t	0.18 ± 0.05
C_{29}	0.03 ± 0.01	0.20 ± 0.03
C_{30}	ND	0.06 ± 0.03
C_{31}	0.01 ± 0.01	0.11 ± 0.05
C_{32}	0.01 ± 0.01	0.11 ± 0.05
C_{33}	ND	0.04 ± 0.01
C_{34}	ND	0.03 ± 0.01
Total	1.37 ± 0.34	4.61 ± 0.79
C.P.I.	24.2	1.83

Alkane values in μg/g dry wt. Abbreviations: C.P.I. = odd/even carbon chain length preference index; ND = not detected: t = trace (< 0.01 μg/g dry wt).

the importance of site selection in future work with biogenic hydrocarbons of coastal marine species.

EXPERIMENTAL

Isolation of non-saponifiable lipids. Frozen kelp blades (60 g fr. wt) were rinsed with MeOH and H_2O, dissected into 2–3 g pieces and saponified (18 hr) in 0.5 M methanolic KOH at 75°. Only young (< 1 month) terminal blades were studied; all were free of epibionts. After cooling to 25°, dilution with H_2O and transfer to a separatory funnel, non-saponifiable lipids were extracted into *n*-hexane, reduced in vol, weighed and stored under N_2.

Column chromatography. Columns (0.9 × 25 cm) containing 5 g Brockman (80–200 mesh) Al_2O_3 over 10 g HiFlosil (60–200 mesh) Si gel were washed by elution with 20 ml C_6H_6, followed by 20 ml *n*-hexane. Non-saponifiable lipids were dissolved in a minimal vol of *n*-hexane and applied to the column. *n*-Alkanes (C_{14}–C_{30}) and monoenes were eluted with 2 × 15 ml *n*-hexane and *n*-alkanes (C_{27}–C_{34}), polyunsaturates, and aromatics were eluted with 2 × 15 ml C_6H_6.

Quantitation. Hydrocarbons were analysed by FID and GLC. A WCOT OV-101 glass column (30 m length and 0.25 mm i.d.) was used. The injector port was maintained at 290° and the detector at 300°. N_2 flow was 1.94 ml/min at 2 kg/cm^2. Samples (0.5–0.7 μl representing 1–5% hydrocarbon in solvent) were injected at 120°, programmed at 3°/min for 50 min and held isothermally at 270° until the C_{34} hydrocarbon eluted from the column (35 min). Peaks were quantified with a computing integrator. Quantiative mixtures of hydrocarbon standards were prepared to verify linear response of the FID and computing integrator, and to identify hydrocarbon constituents. The mixture of unresolved complex hydrocarbons was quantiated by planimetry.

Acknowledgements—The authors are grateful to Dr. Bill Lippincott, Science Applications, Inc., for the collection of kelp samples and to the U.S. Bureau of Land Management for support of this work, under contract No. 008550-CT5-52.

REFERENCES

1. Youngblood, W. W. and Blumer, M. (1973) *Mar. Biol.* **21**, 163.
2. Clark, R. C. and Blumer, M. (1967) *Limnol. Oceanogr.* **12**, 79.
3. Youngblood, W. W., Blumer, M., Guillard, R. L. and Fiore, F. (1971) *Mar. Biol.* **8**, 190.
4. DiSalvo, L. H., Guard, H. E. and Try, K. (1976) *Phycologia* **15**, 245.