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FUCUS 'LIGNIN': A REASSESSMENT*

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Abstract—A 'lignin' fraction isolated by the Björkman method from the marine brown alga *Fucus vesiculosus* has been examined by ^{13}C NMR and degradative analysis, and shown to consist of polyphloroglucinols identical to those characterized earlier from *Fucus*. No evidence for the presence of lignin in *F. vesiculosus* could be found.

INTRODUCTION

Lignins are abundant and universally distributed in woody tissues of gymnosperms and angiosperms, and are present in ferns and club mosses [1–3]. The occurrence of lignin in other taxa of plants is, however, more problematical. Reports of lignins in mosses and liverworts [1, 4–8] have generally not been confirmed by later investigators [9–11], who have found other phenolic cell well components not identical with lignin. Among lower plants, brown algae (Phaeophyceae) are obvious candidates for lignification, as many are large, morphologically complex plants rich in polyphenols [12] and capable of metabolite translocation [13]; however, most early results [1, 14], based on microscopy or elemental analysis, were negative or equivocal, and in any case often suffered interferences from other cellular polyphenols. More recently, various investigators have claimed to demonstrate lignin in quantities from a few percent to nearly 20% of the dry weight of *Cystoseira barbata* [15, 16], *Durvillea antarctica* [17], *Fucus serratus* [4], *F. vesiculosus* [4, 18–20], *Laminaria digitata* [4], and *L. saccharina* [4]. Most of the latter studies have utilized modern analytical techniques, including UV and IR [15, 17–19], chemical degradations [16, 20], and PC, TLC or GC [16, 20].

The present study incorporates three lines of evidence: (i) characterization by ^{13}C NMR and degradative analysis of the 'Björkman lignin' [21] of *F. vesiculosus*; (ii) degradative analysis of the total algal material by the method of Reznikov *et al.* [20]; and (iii) a search for low-MW potential lignin precursors, particularly vanillyl residues.

RESULTS

'*Björkman lignin*' *of* F. vesiculosus

Pretreatment of the *F. vesiculosus* sample (see Experimental) yielded three algal powders: the light brown FR (prepared by freezing and extraction with Et_2O, C_6H_6–EtOH and Me_2CO), the light brown AT (extraction with Me_2CO and toluene), and the dark brown AD (air-dried, extraction with C_6H_6–EtOH and Me_2CO). Subsequent extraction with aqueous dioxane [21] and precipitation from Bu_2O–hexane [18, 19] gave 'Björkman lignin' preparations, respectively light tan, olive green and brownish in colour, in yields of 0.75%, 0.51% and 0.05%. All were soluble in dioxane, EtOH and DMSO; the 'lignins' from FR and AD were also soluble in H_2O and largely soluble in Me_2CO. None was soluble in CH_2Cl_2, $CHCl_3$, hexane, Et_2O, Bu_2O or 1,2-dichloroethane.

^{13}C NMR spectroscopy showed the 'lignin' from AD to consist primarily of mannitol; the 'lignin' from AT was heavily contaminated with bound green tetrapyrrole pigments (red fluorescence under UV light), did not give usable IR or ^{13}C NMR spectra, and was not examined further.

The 'lignin' from FR yielded a ^{13}C NMR spectrum identical to that of the high-MW polyphloroglucinols isolated from *F. vesiculosus* [22; A. G. McInnes, M. A. Ragan, D. G. Smith and J. A. Walter, submitted]. After dialysis, 69.8% of the FR 'lignin' remained, and again yielded a ^{13}C NMR spectrum identical to that of the high-MW polyphloroglucinols (Fig. 1a). Acetylation of 50.0 mg of the nondiffusible fraction from FR yielded 78.8 mg of a slightly coloured product, easily soluble in $CHCl_3$, having a ^{13}C NMR spectrum identical to that of the acetylated *F. vesiculosus* polyphloroglucinols (Fig. 1b).

Methylation of the nondiffusible fraction yielded a brown material soluble with difficulty in pyridine but very poorly soluble in THF, Me_2CO, $CHCl_3$, EtOAc and Et_2O. After Ca–NH_3 degradation, all the coloured material passed readily into EtOAc from acidified H_2O; analysis by GC/MS (Table 1) revealed the fragments expected from Ca–NH_3 degradation of methylated polyphloroglucinols [M. A. Ragan, submitted]. Manual comparison of spectra of all major fragments with spectra of relevant C_6C_2 and C_6C_3 compounds in the 34363-compound EPA/NIH mass spectral data base [23, 24], and computer matching (Incos program LIBRARY) with spectra in the 31331-compound NBS library (Finnigan-MAT No. 20005–30090), revealed no evidence for phenylpropanoid components.

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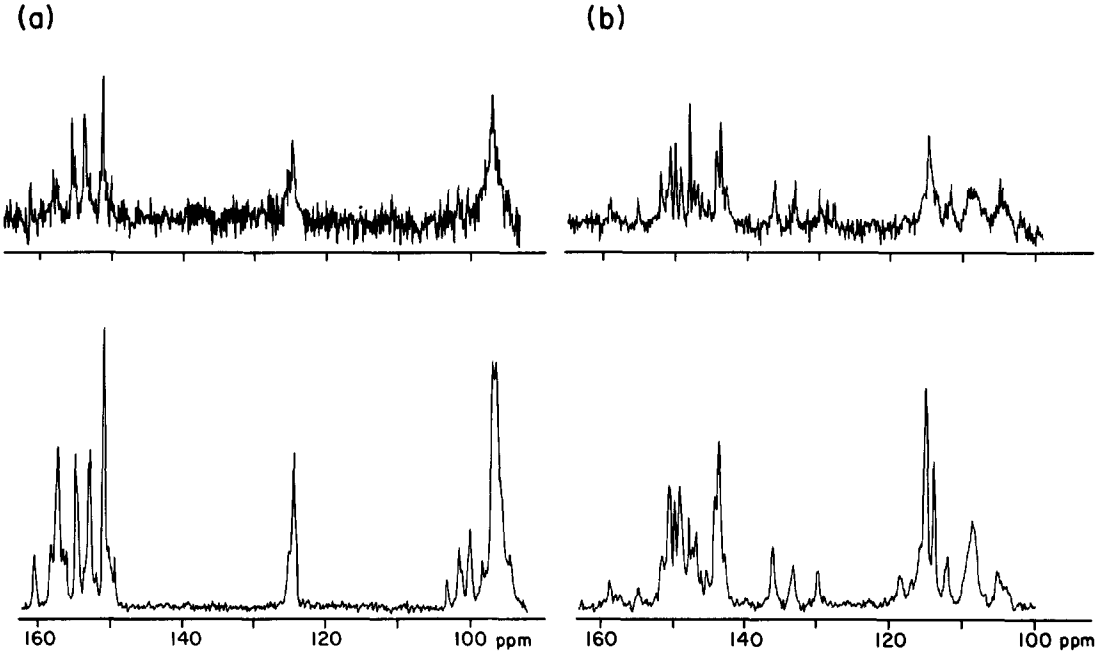

Fig. 1. ^{13}C NMR spectra of FR 'Björkman lignin' from *Fucus vesiculosus* (top) and purified high-MW polyphloroglucinols from *F. vesiculosus* (bottom). (a) Native, underivatized forms; D_2O. (b) Acetoxy derivatives; $CDCl_3$.

Table 1 Compounds identified in Ca–NH_3 degradation product of methylated FR 'lignin' nondiffusible fraction

Relative retention time	MS	Identification
0.425	138, 109	1,3-dimethoxybenzene*
0.579	124, 109	*p*-methoxyphenol
0.598	124	*m*-methoxyphenol*
1.000	168, 139, 125	1,3,5-trimethoxybenzene*
1.161	154, 125, 111	3,5-dimethoxyphenol
2.586	304, 151	2,2′,4,6,6′-pentamethoxybiphenyl*
2939	334, 181, 167	2,2′,4,4′,6,6′-hexamethoxybiphenyl*
4.011	470	octamethoxyterphenyl*
4126	500, 250	2,2′,2″,4,4′,4″,6,6′,6″-nonamethoxyterphenyl*

*Corresponding *C*-methylated derivatives also observed

Degradative analysis of F. vesiculosus

The pretreated algal materials FR and AD (see above) were treated overnight with Na–NH_3 as described by Reznikov *et al.* [20], and the resulting Et_2O-soluble phenolic fractions (FR 0.95%; AD 1.94%) were examined directly by GC/MS. Reference compounds included 4-hydroxy-3-methoxybenzyl alcohol, 3-(4-hydroxyphenyl)-1-propanol, and 4-hydroxy-3-methoxyphenethyl (homovanillyl) alcohol. With the possible exception of traces of phenol, none of the degradation products reported by Reznikov *et al.* [20] was observed.

Low-MW potential lignin precursors in F. vesiculosus

The organic extracts obtained during pretreatment (see above and Experimental) were partitioned into phenolic and nonphenolic fractions, and the former were analysed by TLC in a variety of solvent systems [25]. A number of UV-absorbing compounds were separated, of which many correlated with residual photosynthetic pigments or their degradation products, while many others, readily reactive with acidified vanillin, could be identified as low-MW polyphloroglucinols [26]. Remaining UV-absorbing but vanillin-unreactive compounds were very minor components of these fractions.

The TLC plates were also sprayed with acidified phloroglucinol to screen for vanillin-like metabolites; one major (R_f *ca* 0.2: silica gel, toluene–EtOAc–HOAc, 5:4:1 by vols) and several minor reactive areas were observed, none identical with vanillin (R_f 0.73). The major phloroglucinol-reactive component was partitioned into

CCl_4, and isolated by open-column chromatography (silica gel, Et_2O–hexane to EtOAc–Me_2CO gradient) followed by preparative HPLC (DuPont Zorbax ODS 9.4 mm x 25 cm, MeOH–H_2O gradient). ^{13}C NMR ($CDCl_3$) showed δ_C 173.6, 173.4, 173.2 (fatty acid ester carbonyl), *ca* 13 signals 132.0–127.0 (C=C), 104.1 (β-galactosyl C-1), 74.6, 73.6, 71.2, 70.2, 69.1, 68.2 (glyceryl C-1', C-2'; galactosyl C-2, C-3, C-4, C-5), 63.0 (glyceryl C-3'), 61.8 (galactosyl C-6), and *ca* 21 signals 34.2–14.1 ppm (CH_2 and CH_3); thus the compound is polyunsaturated diacylglyceryl-β-galactolipid(s).

DISCUSSION

The yield of *F. vesiculosus* 'Björkman lignin' described above (to 0.75% of dry matter) is comparable to that reported by Reznikov and Mikhaseva [19] (0.5% of organic matter). However, the 'lignin' isolated in the present investigation was demonstrated by ^{13}C NMR, derivatization and degradative analysis not to be a true lignin, but instead to be the biosynthetically unrelated high-MW polyphloroglucinols [22, 26; A. G. McInnes, M. A. Ragan, D. G. Smith and J. A. Walter, submitted] as earlier speculated [27]. Degradative analysis of the defatted algal powders likewise revealed no evidence of phenylpropanoid components, although this approach is inherently less sensitive than examination of purified 'lignin' fractions, and may not have been sufficiently sensitive to detect fragments released in low yield from minor components of the alga. TLC revealed that most of the organic-soluble UV-absorbing compounds of *F. vesiculosus* which partition into the 'phenolic fraction' are in fact low-MW polyphloroglucinols, or related to photosynthetic pigments. Special attention was given to the possible occurrence of vanillin, claimed [28] to occur in certain older cells of *Fucus*; no evidence for its presence could be found, the only reasonably abundant phloroglucinol-reactive component being galactolipid.

These results call into question the nature of the material extracted and examined by Reznikov *et al.* [18–20]. Published IR spectra of this material [18, 19] bear more similarity to spectra of high-MW polyphloroglucinols (Fig. 2) than to IR spectra of any authentic lignins reviewed by Hergert [29]. The absorption at *ca* 1750 cm^{-1} (C=O stretching) is not observed in all lignins [29], and the bands at *ca* 2900 cm^{-1} (CH_2 asymmetric stretching) could arise from contaminating alkyl compounds (or Nujol). The presence of lignin in *F. vesiculosus*, and by extension in other polyphloroglucinol-containing brown algae [22], must be considered unproven and unlikely.

EXPERIMENTAL

Fresh *Fucus vesiculosus* L. was collected on 19.x.1982 at Fisherman's Beach, Halifax Co., N.S. at low tide, and was transported immediately on ice to the laboratory. The whole plants were cleaned of epiphytes and divided into three batches. FR: 500 g fr. wt were immediately frozen in liq N_2, disintegrated in a Waring blendor, freeze-dried, extracted with Et_2O (1.5 l.) under N_2, Soxhlet-extracted with C_6H_6–EtOH azeotrope (42 hr), air-dried, twice extracted with Me_2CO (N_2, 36 hr), air-dried again, and milled to pass a 40-mesh screen. AT: 400 g fr. wt were thrice extracted with 2700 ml Me_2CO (N_2), dried *in vacuo* (25°), milled (20 mesh), extracted with Me_2CO (2 l.) then toluene (2 l.), air-dried and milled (40 mesh). AD: several kg were air-dried (5 days), milled (20 mesh), Soxhlet-extracted with C_6H_6–EtOH azeotrope (42 hr), air-dried, ground, extracted with Me_2CO (2 l.), dried *in vacuo* (50°), then milled (40 mesh). All three powders were then dried *in vacuo* over P_2O_5 (45°, 93 hr). Aliquots (70–100 g) were stirred with 4% aq. dioxane (2 successive extractions of 44 and 20 hr, 200–250 ml), filtered, the filtrates concentrated *in vacuo* (37°), redissolved in dioxane (5 ml), precipitated from Bu_2O–C_6H_6 (100:5 v:v) [18, 19], and the ppts washed (Bu_2O–C_6H_6) and dried *in vacuo* (25°).

^{13}C NMR spectra (20.11 MHz) were recorded in D_2O (ref: dioxane) or $CDCl_3$ (ref: TMS) on a Varian FT-80. IR spectra (film on AgCl) were recorded on a Perkin–Elmer 283B. GC/EIMS (70 eV) was carried out at the New Brunswick Research and Productivity Council (Fredericton) on a Finnigan 4021, with chromatography on a DB-1 fused silica column (30 m, 0.3 mm i.d.), He carrier, splitless injection (50°, hold 2 min) and sub-

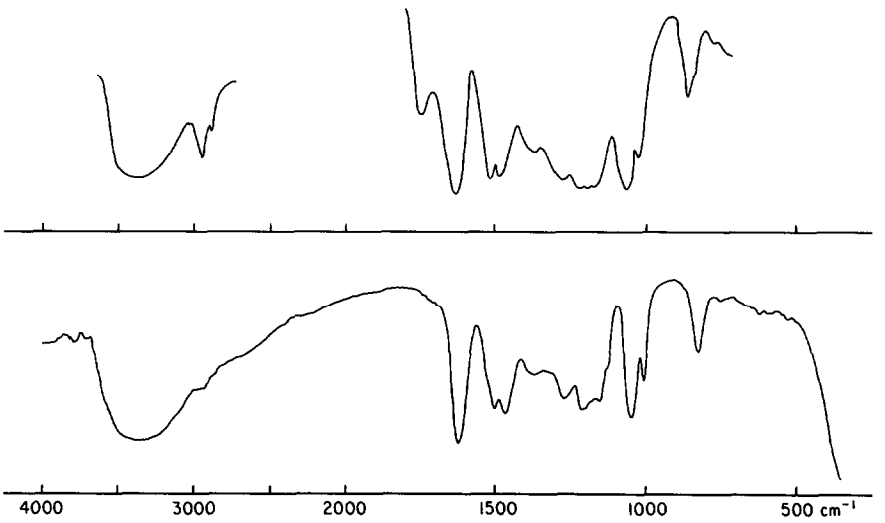

Fig. 2. Top: IR spectrum reported for 'lignin' from *Fucus vesiculosus* (redrawn from ref. [18]). Bottom: IR spectrum of purified high-MW polyphloroglucinols from *F. vesiculosus* [M. A. Ragan, submitted].

sequent programming from 75 to 275° (hold) at 8°/min. Acetylation (Ac_2O–NaOAc), methylation (Sjöberg/Hakomori) and Ca–NH_3 degradation have been described elsewhere [M. A. Ragan, submitted].

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