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WIDESPREAD DISTRIBUTION OF SULFATED POLYPHENOLS IN BROWN ALGAE

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INTRODUCTION

It has been recognized for a number of years that the marine brown alga *Ascophyllum nodosum* contains a particularly labile, low MW polyphenol [1, 2] in addition to the higher MW polyphloroglucinols characteristic of many Phaeophyceae [3, 4]. This labile substance was termed 'phenolic precursor' because it was thought to give rise to high MW polyphenols in extracts or exudates from *A. nodosum* [5]; its possible occurrence in brown algae other than *A. nodosum* could not be ascertained due to the large quantities of polyphenols, mannitol and salts which interfered with most chromatographic separations. The recent identification of the *A. nodosum* 'phenolic precursor' as 1,2,3,5-tetrahydroxybenzene 2,5-disulfate ester (**1**) [6] suggested paper electrophoresis at low pHs to be the analytical method of choice. We now report the results of a survey of 20 species of brown algae (Phaeophyceae), representing 7 orders and 10 families (Table 1).

RESULTS AND DISCUSSION

Two vanillin-reactive, mobile zones were seen on electrophoretograms of extracts from many brown algae (Table 1): a faster-moving zone, giving a positive (reddish-pink) but slowly developing colour with the vanillin reagent, co-electrophoresing with and indistinguishable from authentic **1**; and a slower zone, reacting more rapidly than **1** but more slowly than the uncharged phloroglucinols, indistinguishable from 1,2,3,5-tetrahydroxybenzene 2-sulfate ester (**2**). At pH 4.00, the mobilities relative to phloroglucinol trisulfate ester were approximately 0.76 and 0.56, respectively [7].

These vanillin-reactive, electrophoretically mobile zones could be demonstrated in electrophoretograms of extracts from representatives of at least 6 orders of Phaeophyceae, from the most simple morphologically (Ectocarpales, where only trace quantities were found) to the most complex (Fucales). The complete absence of extractable vanillin-reactive material from *Desmarestia viridis* may be an artefact arising from the unusually low intracellular pH in this species [8]. No vanillin-reactive materials could be detected in *Mycosphaerella ascophylli*, the fungus naturally endophytic in *A. nodosum* and *Pelvetia canaliculata* [9], nor in the brownish-coloured Rhodophycean alga *Dumontia incrassata*.

Two-dimensional PC corroborated the presence of the **1**-like compound in *A. nodosum*, *Fucus serratus*, *F. vesiculosus*, *P. canaliculata*, *Laminaria saccharina*, and possibly *L. hyperborea*, and furthermore suggested its presence in *F. spiralis* and *F. distichus* ssp. *edentatus* ('*F. inflatus*').

The apparently widespread distribution of sulfated polyphenols among brown algae reinforces the suggestion [10] that the occurrence of such sulfate esters may

Table 1. Distribution in Phaeophyceae of compounds indistinguishable from **1** and **2**

Alga	1-Like	2-Like
ECTOCARPALES: Ectocarpaceae		
Pilayella littoralis (L.) Kjellm.	tr*	—
Spongonema tomentosum (Huds.) Kütz.		
Chordariaceae	tr†	tr
Chordaria flagelliformis (O. F. Müll.) C.Ag.	—	tr‡
DICTYOSIPHONALES: Punctariaceae		
Punctaria sp.	—	—
SCYTOSIPHONALES: Scytosiphonaceae		
Petalonia fascia (O. F. Müll.) Kuntze	+	+
Scytosiphon lomentaria (Lyngb.) Link	+	+
DESMARESTIALES: Desmarestiaceae		
Desmarestia viridis (O. F. Müll.) Lamour.	—	—
LAMINARIALES: Chordaceae		
Chorda filum (L.) Stackh.	+	+‡?
Laminariaceae		
Laminaria digitata (Huds.) Lamour.	tr	+‡
L. hyperborea (Gunn.) Foslie	tr?	+‡
L. saccharina (L.) Lamour.	tr	+‡?
Alariaceae		
Alaria esculenta (L.) Grev.		
thallus	—	—
sporophylls	tr	—
DICTYOTALES: Dictyotaceae		
Dictyota dichotoma (Huds.) Lamour.	++	+
FUCALES: Fucaceae		
Ascophyllum nodosum (L.) LeJol.	++	+
Fucus distichus (L.) ssp. *anceps* (Harv. et Ward ex Carr.) Powell	+	—
F. serratus (L.)	++§	+‡
F. vesiculosus (L.)		
high-littoral form	++	+
mid-littoral (typical) form	++	+
Pelvetia canaliculata (L.) Dcne. et Thur.	++	+‡?
Himanthaliaceae		
Himanthalia elongata (L.) S. F. Gray		
receptacles	—	+
vegetative thallus	tr	+

* ++, > approx. 200 μg/g fr. wt; +, approx. 10–200 μg/g fr. wt; tr, approx. 5–10 μg/g fr. wt. (at limit of detection).

† Relative mobility (0.80) slightly greater than expected.

‡ Also traces of slower-moving, vanillin-reactive compounds in these species.

§ Possibly two compounds in this zone.

be correlated with adaptations to an aquatic habitat. Although the faster and slower zones observed on the electrophoretograms were indistinguishable from **1** and **2** respectively, unambiguous identification must await definitive evidence from ^1H NMR and ^{13}C NMR spectroscopy in each case. The difficulties presently associated with isolation and purification of sufficient material for spectroscopic investigation point out the need for better preparative methods for the study of these compounds.

EXPERIMENTAL

Pelvetia canaliculata and *A. nodosum* were collected at Østmarkneset, Trondheim; the other algae, plus additional *A. nodosum*, were collected at Golten (Sotra) and Espeland (Blomsterdalen) near Bergen. All collections were made in May and June, 1978.

Samples (2–3 g fr. wt) were extracted with aq. Me_2CO [11], then filtered (Whatman GF/C). Me_2CO was removed *in vacuo*, and the aq. phase was filtered (GF/C), lyophilized and redissolved in H_2O (2.0 ml); aliquots (10–200 μl) were analysed by paper electrophoresis at pH 1.80 (glycine–HCl buffer) and at pH 4.00 (acetate buffer) (2 hr, 6 V/cm), as well as by 2D PC (Whatman No. 1 paper; solvents *n*-BuOH, then 20% (w/v) aq. KCl). Compounds were detected by spraying the dried chromatograms or electrophoretograms with vanillin–HCl, then heating gently. Semiquantitative estimates were made using known concentrations of **1** as the standard; due to problems of lability and possibly incomplete extraction, the estimates in Table 1 are probably low.

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