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ALTRITOL IN THE BROWN ALGA *HIMANTHALIA ELONGATA*

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Abstract—Natural abundance ^{13}C NMR spectroscopy has shown that altritol (talitol) is a major constituent within vegetative and reproductive tissues of *Himanthalia elongata*. Quantitative GC studies have confirmed that altritol concentrations are 80–160% higher than for the hexitol mannitol (which occurs throughout the Phaeophyta). The data suggest an osmotic role for altritol.

INTRODUCTION

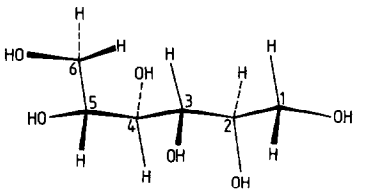
The hexitol D-mannitol is a major intracellular constituent of marine brown algae, representing up to 20% of the dry wt of thalloid forms [1]. Its occurrence is generally regarded as taxonomically important; all members of the Phaeophyta which have been studied to date show photoassimilation of ^{14}C into mannitol [2, 3]. The only other low MW carbohydrate reported from a marine brown alga is the heptitol volemitol (D-glycerol-D-mannoheptitol) which occurs in the topshore alga *Pelvetia canaliculata*. Although *P. canaliculata* grows characteristically in symbiosis with a fungal endophyte, *Mycosphaerella* sp., detailed studies have shown that volemitol is a product of algal, rather than fungal, metabolism [4, 5].

In this paper, we report the novel occurrence of the hexitol altritol (talitol) in the lower shore macroalga *Himanthalia elongata*, an alga which occurs most frequently on exposed shores [6]. Mature plants of *H. elongata* grow as button-like thalli, up to 6 cm in diameter, with long strap-shaped receptacles arising from the centre of the frond, hence its common name—'thong-weed'. The present study forms the first such report of altritol from a biological source.

RESULTS AND DISCUSSION

A survey of the low MW carbohydrates of marine brown macroalgae (using natural abundance ^{13}C NMR spectroscopy) revealed that samples of both vegetative and reproductive tissues from *H. elongata* contained, in addition to mannitol, a further low MW compound. Intact plant tissues and ethanol extracts showed eight resonances at δ63.6, 64.5, 64.6, 70.7, 71.9, 72.2, 73.0 and 74.0.

Three of these resonances correspond to the ^{13}C spectrum of mannitol (i.e. δ64.6, C-1, C-6; 70.7, C-3, C-4; 72.2, C-2, C-5) [7]. However, in samples of *H. elongata*, the resonance at δ72.2 is too intense to be accounted for by mannitol alone. It can, however, be accounted for by the superposition of a second resonance at δ72.2 which, together with the remaining absorptions at δ63.6, 64.5, 71.9, 73.0, 74.0 are in close agreement with the published values for altritol (δ64.4, C-1; 71.8, C-2; 72.2, C-3; 73.0, C-4; 74.0, C-5; 63.5, C-6), but not for any other alditol [7].



GC separation of TMSi derivatives using the procedures of Holligan and Drew [8] gave a peak with an identical R_t to altritol (1.12 relative to α-glucose) [8], although mannitol (R_t 1.09 relative to α-glucose) appeared as a shoulder on the leading edge, due to incomplete separation. Using a modified programme, with a lower initial temperature and slower rate of temperature increase, TMSi derivatives of mannitol and altritol could be quantified individually.

Samples of *H. elongata* were collected from two sites on the East coast of Scotland—at Arbroath and Fife Ness; vegetative and reproductive tissues were analysed separately using GC (Table 1). In all cases, altritol concentrations were higher than those for mannitol. Samples from Fife Ness contained greater amounts of both altritol and mannitol than those from Arbroath with vegetative tissues from Fife Ness showing highest accumulation of both hexitols. Receptacles from *H. elongata* at Fife Ness contained both altritol and mannitol at concentrations which are significantly lower than those of vegetative tissues (*t*-tests; $P = 0.01$) from the same source. Vegetative and reproductive tissues from *H. elongata* at Arbroath contain similar quantities of altritol and mannitol (Table 1).

The hexitol concentrations shown in Table 1 represent osmotically significant levels of both altritol and mannitol; the altritol concentration within cells of vegetative thalli from Fife Ness is sufficient to balance approximately

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Table 1. Intracellular hexitol concentrations of *Himanthalia elongata*

	Fife Ness* (mmol/kg)†		Arbroath* (mmol/kg)†	
	Mannitol	Altritol	Mannitol	Altritol
Vegetative tissue	140.9 ± 19.7	359.2 ± 24.2	40.1 ± 3.7	100.4 ± 32.5
Reproductive tissue	65.9 ± 8.7	121.8 ± 21.1	47.8 ± 6.7	87.9 ± 7.4

*Five replicates of each tissue were assayed (mean + standard deviation).

†Values are expressed in terms of intracellular water content (69.9% and 76.8% fr. wt for vegetative and reproductive tissues respectively).

one-third of the total external salt concentration (sea water osmolality was measured at 1040 mosmol/kg). Coupled with ^{13}C NMR observations of intact *H. elongata*, which showed no evidence of any other resonances due to other organic solutes, the data strongly suggest an osmotic role for altritol. Similar conclusions have been made for the heteroside glucosylglycerol in the marine blue–green alga *Synechocystis* sp. [9, 10] and the quaternary nitrogen compound glycine betaine in the halophilic bacterium *Ectothiorhodospira halochloris* [11], based on data from natural abundance ^{13}C NMR spectra.

Further evidence of the osmotic role of altritol and mannitol in *H. elongata* is contained within Table 2. Intracellular K^+ concentrations show variations which are the inverse of those shown in Table 1 for the hexitols (altritol + mannitol). K^+ forms the dominant cationic component within cells of most marine algae [12, 13], and intracellular K^+ levels are lowest in samples of *H. elongata* containing greatest amounts of hexitols (i.e. in vegetative tissues from Fife Ness) and *vice versa* (highest K^+ levels were recorded in reproductive tissue from Arbroath). While an osmotic role has been proposed for mannitol in marine brown algae [14, 15] the present study represents the first report of an osmotic role for altritol.

EXPERIMENTAL

H. elongata (L.) S. F. Gray was collected during June–July, 1983 from the lower intertidal zone at Fife Ness, Fife, Scotland (National Grid Ref. No 638098) and from Arbroath, Angus, Scotland (National Grid Ref. No 659412); specimens from each site have been placed within the herbarium collection of the University of Dundee. Natural abundance ^{13}C $\{^1H\}$ NMR spectra were measured using a f.t. spectrometer, operating at 15.08 MHz. 10% D_2O was added to each sample to provide a lock signal and MeOH (δ49.0 downfield from TMS) was used as int. standard.

Spectra were obtained using a 7 μs pulse (65°) and a recycle time of 1.1 sec. A total of 1500 scans was accumulated for each sample; these were transformed after exponential broadening of 1.5 Hz. Samples were assayed at 30° in 10 mm o.d. tubes, using either (a) intact portions of thallus or receptacle, compacted into the lower 5 cm of the tube, together with a soln of NaCl (3.5% w/v), or (b) concd extracts of plant material (30–32 g fr. wt) in 2 cm^3 80% EtOH [8].

GC separations of altritol and mannitol were carried out following extraction in 80% EtOH (0.15–0.25 g in 10 cm^3), and derivatization, as described previously [8, 12, 15]. Samples were analysed on a 2 m column containing 2% SE52 on a diatomite support, fitted to a FID instrument. A temp prog from 90° to 160° at 4° min was used, with a final (isothermal) phase at 160° for 10 min. All other conditions were as described previously [8]. Arabitol (2 mg) was used as int. standard; peak areas were quantified using an electronic integrator.

Intracellular H_2O content was assayed as fr. wt–dry wt–extracellular H_2O [15]. Dry wts were obtained following a period of 96 hr at 65°; extracellular H_2O contents were determined using ^{14}C sorbitol, as described previously [15]. K^+ analyses were conducted, following a 5 min rinse in isotonic $Ca(NO_3)_2$ to remove extracellular cations from the cell wall matrix, using a flame emission spectrophotometer [16].

Table 2. Intracellular K^+ concentrations of *Himanthalia elongata*

	Fife Ness* (mmol/kg)†	Arbroath* (mmol/kg)†
Vegetative tissue	221.0 ± 17.4	291.4 ± 16.9
Reproductive tissue	242.5 ± 9.6	303.7 ± 13.5

*Three replicates of each tissue were assayed (mean + standard deviation).

†Values expressed in terms of intracellular water content, as given in Table 1.

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