European Journal of Phycology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713725516

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To cite this Article Reed, Robert H.'Osmoacclimation in *Bangia atropurpurea* (Rhodophyta, Bangiales): the osmotic role of floridoside', European Journal of Phycology, 20: 3, 211 — 218
To link to this Article: DOI: 10.1080/00071618500650221
URL: http://dx.doi.org/10.1080/00071618500650221

Br. phycol. J. **20:** 211–218
1 September 1985

Osmoacclimation in *Bangia atropurpurea* (Rhodophyta, Bangiales): the Osmotic Role of Floridoside

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Aspects of the osmotic physiology of freshwater-grown plants of the euryhaline alga *Bangia atropurpurea* (Roth) C. Ag. have been studied over the salinity range from freshwater to full-strength sea-water (35‰). Synthesis of floridoside [O-α-D-galactopyranosyl-(1→2)-glycerol], the major low molecular weight organic solute in *B. atropurpurea*, occurred upon transfer of plants from a freshwater-based medium to saline media, with a maximum level of 134 mmol kg (cell water)$^{-1}$ being recorded for thalli incubated in 100% sea-water for 24 h. In contrast, photosynthetic activity was depressed in high-salt media. Differences in the kinetics of floridoside production and photosynthetic rate were also observed during transfer to 100% sea-water; floridoside showed a rapid increase, doubling in concentration within 30 min and reaching a new elevated steady state within 24 h while photosynthetic activity showed a transient decrease of approximately 75% upon transfer, followed by a recovery to 60% of the initial (freshwater) rate. Studies using ^{14}C showed an increasing allocation of radiocarbon to floridoside with increasing external salt concentration, with a six-fold increase in the percentage of ^{14}C incorporated into this heteroside between freshwater and sea-water. Overall, the data are consistent with the hypothesis that changes in floridoside concentration serve to compensate, in part at least, for changes in external osmolality.

The heteroside O-α-D-galactopyranosyl-(1→2)-glycerol, or floridoside (Colin & Guéguen, 1930; Augier & Du Merac, 1954) is widely distributed among the members of the Rhodophyta, with levels up to 10% of tissue dry weight in some marine thalloid forms (Reed, Collins & Russell, 1980a). Floridoside occurs only in those red algae that do not belong to the Ceramiales [where digeneaside (O-α-D-mannopyranosyl-(1→2)-glycerate) replaces floridoside as the major low molecular weight carbohydrate (Colin & Augier, 1939; Kremer, 1981)]. Members of the Bangiales may also contain an isomeric form (linked 1→1), termed isofloridoside. However, ^{14}C-labelling studies have shown that floridoside is the principal photoassimilated compound, with isofloridoside consistently showing rather weak ^{14}C-labelling (Craigie, McLachlan & Tocher, 1968). Thus the two forms of galactosyl–glycerol are not equivalent as metabolites in the Rhodophyta, with floridoside acting as the metabolically active photoassimilatory product.

The involvement of floridoside in osmotic adjustment was first suggested for marine red algae by Kauss (1968, 1969), who showed that the extent of ^{14}C-labelling of galactosyl–glycerols in *Iridophycus* and *Porphyra* could be altered by changing the salinity of the incubation medium. Thus galactosyl–glycerol synthesis was strongly induced by high external salt concentrations, while incubation in low-salt media led to a substantial reduction in galactosyl–glycerol synthesis (Kauss, 1968). Earlier studies had clearly established an osmotic role for isofloridoside in the freshwater Chrysophyte *Poterioochromonas*, with ^{14}C-labelling patterns and intracellular isofloridoside levels showing a direct relationship to the external salinity (Kauss, 1967a, b). In a series of subsequent papers, Kauss and co-

0007-1617/85/030211+08 $03.00/0

workers have investigated in detail the control of isofloridoside synthesis and degradation and the effects of external salt status upon these processes, showing that the enzymes responsible for the interconversion of isofloridoside and reserve polysaccharide may be directly sensitive to changes in external osmolality (see Kauss, 1978). Despite these detailed studies of isofloridoside metabolism in *Porterioochromonas*, less research has been carried out using red algae and the osmotic role of floridoside remains the subject of some controversy.

In a study of the effects of salinity upon photoassimilatory products in several marine red algae, including *Porphyra*, Kremer (1979) was unable to detect any substantial effects of external salt concentration upon either ^{14}C-incorporation or thallus levels of floridoside following short-term incubation (60 min) in hyposaline and hypersaline media. He concluded that the biosynthesis of this heteroside remains unaffected by osmotic stress over a wide range of salinity and that osmotic adjustment in marine red algae is not achieved by varying the intracellular level of floridoside. On the basis of these observations, Kremer (1979) has suggested that floridoside in marine red algae does not behave in a comparable manner to isofloridoside in *Porterioochromonas* but rather that it functions as a photoassimilatory product and as a source of carbon for respiration (Kremer, 1981). However, several recent studies have shown that floridoside levels may be affected by changes in salinity, in accordance with the hypothesis that galactosyl–glycerols may function as osmotic effectors in red algae. Kirst & Bisson (1979) have shown that the percentage of ^{14}C-labelling of floridoside in *Grateloupia* and *Hypnea* is sensitive to changes in salinity and that intracellular floridoside concentration in *Grateloupia*, *Hypnea*, *Corallina*, *Lomentaria* and *Rhodymenia* is affected in a similar manner. Comparable results have also been obtained for *Porphyra purpurea* (Roth) C. Ag. (Reed *et al.*, 1980a) and *P. umbilicalis* (L.) J. Ag. (Wiencke & Läuchli, 1981). The acidothermophilic unicell *Cyanidium caldarium* (Tilden) Geitler also accumulates floridoside when subjected to osmotic stress, confirming its classification within the Rhodophyta and adding further support to the hypothesis that floridoside is involved in osmotic adjustment (Reed, 1983a). Such observations conflict with the suggestion of Kremer (1981) that the intracellular concentration of floridoside and other low molecular weight carbohydrates passively follows external osmotic fluctuations, rather than participating in (active) osmoacclimation.

The present study was undertaken to try to resolve some of the controversy regarding the osmotic role of floridoside in red algae, using the euryhaline alga *Bangia atropurpurea*. This alga grows both in freshwaters and sea-water and was once regarded as two distinct species (see Geesink, 1973). Previous studies have shown that *B. atropurpurea* collected from a freshwater location can survive and grow upon transfer to saline media, at least up to full-strength sea-water (Reed, 1980a), suggesting that freshwater populations of this alga may be regarded as euryhaline ecotypes of the marine form. The effects of upshock (increasing external salinity) upon intracellular floridoside concentration and photosynthetic rate have been established, to see whether galactosyl–glycerol levels are governed solely by photosynthetic activity (Kremer, 1979) or rather by external osmolality (Kauss, 1968). The transfer of algal material from a freshwater-based medium to a series of saline media counters one of the objections raised by Munda & Kremer (1977) who suggested that the decreased levels of low molecular weight carbohydrates observed when marine algae are subjected to hypoosmotic stress (downshock) are due to the combined effects of photosynthetic impairment in hyposaline media, coupled with an increased respiration rate. Freshwater *B. atropurpurea* grows best in freshwater and extreme hyposaline media (Reed, 1980a), showing maximum rates of photosynthetic activity under these con-

ditions (Sheath & Cole, 1980), and thus any increases in floridoside level upon upshock could not be dismissed as "passive".

MATERIALS AND METHODS

Bangia atropurpurea was collected from an overflow channel on the Leeds–Liverpool Canal at Wigan (Reed, 1980a) and transported to Dundee in a sealed polystyrene container filled with ice. Individual thalli were then separated from all other plants using forceps and subsequently transferred to filtered canal water (collected from the same site). Thalli were maintained at 8°C for 24 h under continuous illumination (cool-white fluorescent lighting at a photon fluence rate of 25 μmol m^{-2} s^{-1}) prior to experimentation. Samples were then transferred to filtered canal water at 20°C for 3 h, to minimize the effects of temperature change. All experiments were carried out at 20°C, under continuous illumination (at a photon fluence rate of 45 μmol m^{-2} s^{-1}) unless otherwise stated in the text.

Since *B. atropurpurea* from the Leeds–Liverpool Canal shows poor growth and survival in all freshwater-based media containing distilled, deionized water, sterile canal water enriched with ES nutrients and trace metals (McLachlan, 1973) was used as the basis for a freshwater medium (Reed, 1980a). Hyposaline media, based on the ASP12S medium described by Reed, Collins & Russell (1980b), were formulated to give final salinities corresponding to 10, 25, 50, 75% and full-strength (100%) sea-water (at a salinity of 35‰); plants were incubated in each of these media for up to 48 h, with forced aeration. $NaHCO_3$ was added to a final concentration of 4 mmol dm^{-3} in all media.

Cellular water content was estimated according to the following relationship: cell water content = fresh weight − dry weight − extracellular water. Fresh weight was determined after removal of excess moisture from samples of algal material by vacuum filtration (Hall, 1981); dry weight was determined using samples maintained at 100°C for 48 h; extracellular water content was determined by short-term incubation studies using [^{14}C]-sorbitol (Reed, 1980b).

Samples of plant material of known fresh weight (30–50 mg) were analysed for their floridoside content following extraction in boiling 80% ethanol and subsequent incubation in 80% ethanol at room temperature for 18 h. Extracts were then dried using a rotary evaporator and stored *in vacuo* for 48 h prior to trimethylsilyl derivatization (Reed *et al.*, 1980a). Samples were analysed using a Varian 3700 gas–liquid chromatograph, equipped with a flame ionization detector and fitted with a 2 m column (internal diameter 4 mm), containing 2% SE52 methylphenylsilicone gum on a diatomite support. A temperature programme, from 140 to 280°C at 20°C min^{-1}, was used, holding the initial and final temperatures for 2 min. All other chromatographic conditions were as described previously (Reed *et al.*, 1980a). Arabitol was used throughout as an internal standard, being added at the first 80% ethanol extraction step.

Rates of net photosynthesis were obtained using a polarographic technique (Reed, Collins & Russell, 1979). Samples of algal material (up to 20 mg fresh weight) were incubated in the chamber of an O_2 electrode (Rank Bros., Bottisham, Cambridge, UK) containing 5 cm^3 of experimental medium at 20°C and at a saturating, but not inhibitory, photon fluence rate of 250 μmol m^{-2} s^{-1}, incident upon the outer surface of the electrode chamber. Media were sparged with N_2 to reduce their O_2 content to approximately 50% of the air-equilibrated value, to ensure that O_2-saturation was not reached during analysis of photosynthetic activity. Algal material was allowed to photosynthesize for up to 10 min, to obtain steady rates of photosynthetic O_2 evolution. All other conditions were as given by Reed *et al.* (1979).

Selected thalli (30–40 mg fresh weight in total) were allowed to photosynthesize in incubation media containing ^{14}C-labelled $NaHCO_3$ (at specific activities between 30–50 GBq mol^{-1}) at a photon fluence rate of 250 μmol m^{-2} s^{-1} for a total of 30 min. Plant material was then removed and freed of the adhering medium by vacuum filtration (Hall, 1981). Thalli were then rinsed briefly (20–30 s) in a medium of identical composition, but without added ^{14}C tracer and transferred to boiling acidified 80% ethanol at pH 2·0. Two subsequent extraction steps were carried out, by incubating (at 20°C) in acidified 80% ethanol for 24 h in each case. The remaining solid material was then removed and assayed for ^{14}C activity in 5 cm^3 of Packard 299 scintillation cocktail (Packard Instruments, Illinois, USA), using a Packard 300 liquid scintillation spectrometer with automatic quench correction (sample channels ratio); this fraction represents the storage and structural component of ^{14}C photoassimilation in *B. atropurpurea*.

All of the 80% ethanol extracts for any given sample were pooled to provide a low molecular weight carbohydrate fraction; this was evaporated to dryness using a rotary evaporator and redissolved in 100 mm^3 of distilled water. Aliquots (10–25 mm^3) were then transferred to cellulose plates (Eastman Kodak, Kirkby, Merseyside, UK) prior to thin-layer chromatography using butanol : pyridine : glacial acetic acid : water (60 : 40 : 3 : 30) (Reed *et al.*, 1980a),

with authentic floridoside as a marker. The amount of ^{14}C associated with the floridoside component was assayed following removal of the appropriate portion of the chromatogram and transferrence to scintillation fluid. Measurement of the ^{14}C content of the remainder of the chromatogram provided data for all other low molecular weight carbohydrates (see Kirst & Bisson, 1979; Kremer, 1979).

RESULTS

The effects of salinity upon floridoside concentration and photosynthetic activity

Data for the intracellular water content of *B. atropurpurea* maintained in a range of salinities for 24 h are shown in Fig. 1(a). Intracellular water content showed an inverse relationship to external salt concentration with maximum values in fresh water. However, the overall change in cell water content was found to be less than 8% across the entire range. In this respect, *B. atropurpurea* responds in a similar manner to *Porphyra* (Reed *et al.*, 1980b), *Pilayella* (Reed & Barron, 1983) and *Polysiphonia* (Reed, 1983b), since all of these algae show a progressive reduction in cell water content with increasing salinity, due to the combined effects of increasing percentage dry weight and extracellular water content.

The values for intracellular water content have been used to express both the floridoside level and the rate of net photosynthesis in terms of cellular water content, to make the results more directly comparable. Intracellular floridoside concentration was found to increase dramatically following 24 h incubation in all saline media [Fig. 1(b)], with maximum levels [134 mmol kg (cell water)$^{-1}$] in full-strength sea-water medium. This represents an increase of approximately 10-fold when compared to algal material maintained in freshwater [Fig. 1(b)], showing clearly that the intracellular concentration of floridoside is sensitive to the external salt level. Gas–liquid chromatographic analysis showed that isofloridoside remained as a minor component [<2 mmol kg (cell water)$^{-1}$] throughout the course of the present study and its osmotic significance will thus not be considered further.

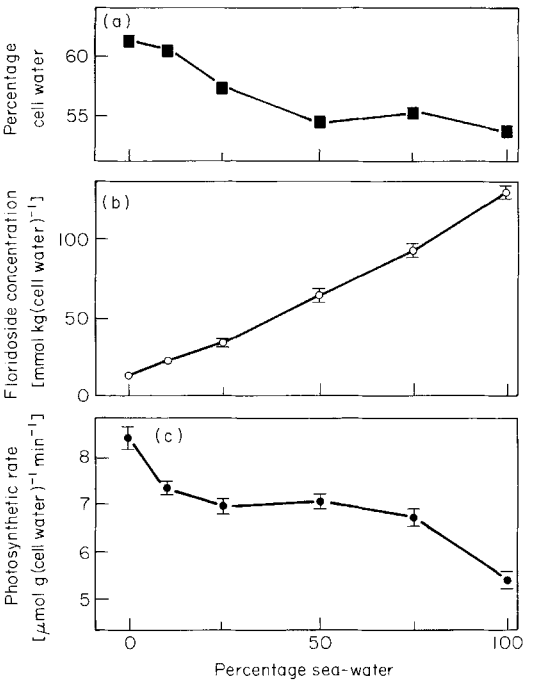

FIG. 1. The effects of salinity upon (a) cellular water content (■, two replicates); (b) intracellular floridoside concentration (○, three replicates); and (c) photosynthetic O_2 evolution (●, two replicates) in *Bangia atropurpurea*, following a 24-h incubation period in a range of saline media up to 100% sea-water. S.D. values shown, as appropriate, unless S.D. < symbol size.

Figure 1(c) shows that photosynthetic activity was greatest in thalli maintained in fresh water with a somewhat lower value between 10 and 75% sea-water, while incubation in 100% sea-water led to a further sharp decline in photosynthetic rate. However, the value in full-strength sea-water was greater than 60% of the rate in fresh water, showing that the photosynthetic competence of this alga is not completely disrupted upon transfer from freshwater to sea-water. It is also worth noting that the effects of elevated salinity upon photosynthetic activity contrast sharply with the changes in floridoside concentration [Fig. 1(b)].

Temporal changes in floridoside concentration and photosynthetic activity upon transfer to full-strength sea-water

A time course for the changes in floridoside concentration in cells of *B. atropurpurea* transferred from freshwater to 100% sea-water for up to 48 h is shown in Fig. 2(a). The intracellular level of floridoside showed a rapid increase upon upshock, doubling in less than 30 min and reaching a new steady state within 24 h. Changes in intracellular floridoside level occur within a similar time scale in *Porphyra* (Reed *et al.*, 1980a).

In contrast, photosynthetic activity declined sharply upon upshock to full-strength sea-water, being reduced by more than 75% at 2 min [Fig. 2(b)]. However, photosynthetic activity rapidly rose to achieve a new steady state within approximately 1–2 h. This value, representing approximately 60% of the activity in fresh water, is comparable to the rate shown in Fig. 1(c) for cells in 100% sea-water medium.

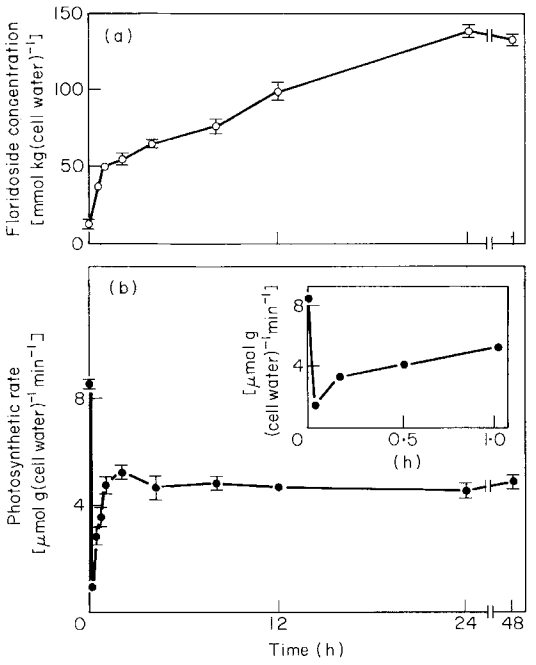

FIG. 2. Time courses for (a) floridoside concentration (○, three replicates) and (b) net photosynthesis (●, two replicates) following transfer of *Bangia atropurpurea* from freshwater to full-strength sea-water. Insert, Fig. 2(b) shows details of short-term changes in photosynthetic O_2 evolution, up to 1 h. S.D. values shown, unless S.D. < symbol size.

^{14}C-Labelling of floridoside and the effects of salinity upon ^{14}C partitioning in *B. atropurpurea*

Figure 3(a) shows that the percentage of ^{14}C incorporated into low molecular weight compounds increased dramatically with increasing salinity. Thus, after 30 min incubation in $NaH^{14}CO_3$-containing sea-water, more than 98% of the radioactivity was found to be associated with the 80% ethanol fraction, whereas thalli maintained

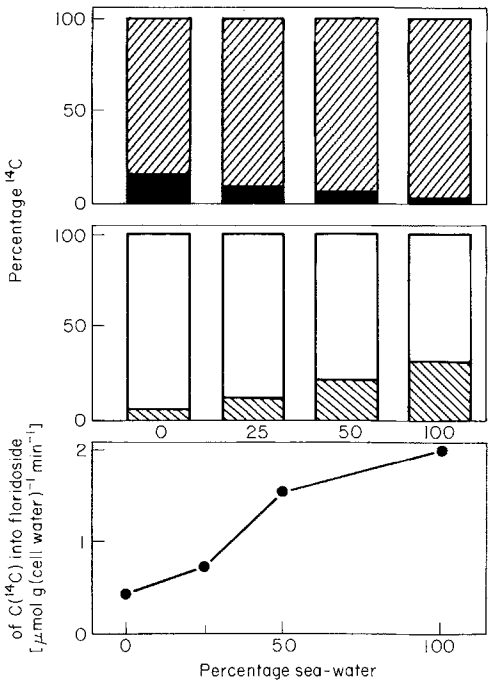

FIG. 3. Radiocarbon-labelling patterns of *Bangia atropurpurea* following a 24-h pre-equilibration period in either freshwater, or 25, 50 or 100% sea-water. The relative partitioning of total ^{14}C activity (30-min incubation period) into low molecular weight carbohydrates (▨) and storage plus structural compounds (■) is shown in (a). The percentage of ^{14}C within the low molecular weight fraction that was assimilated into floridoside is shown in (b) (▧), together with the remaining fraction of ^{14}C activity (□). Rates of incorporation of ^{14}C into floridoside (●) are shown in (c). Values represent the means of two replicate samples in all cases.

in freshwater showed 86% of ^{14}C in the same fraction. The proportion of radiocarbon being channelled into structural and storage compounds (the ethanol-insoluble component) in plant material incubated in sea-water was thus lower than that of freshwater-incubated plants by approximately seven-fold, despite the limited reduction in photosynthetic activity which accompanied the transfer to 100% sea-water [Fig. 1(c)].

The percentage of radioactivity (from the low molecular weight fraction) associated with floridoside was found to increase with salinity [Fig. 3(b)], with a six-fold increase across the salinity range from freshwater to sea-water. Similarly, when the combined data were used to calculate the incorporation of inorganic carbon into floridoside, as shown in Fig. 3(c), a clear trend of increasing incorporation with increasing external salt concentration was observed, with a five-fold increase from freshwater to 100% sea-water.

DISCUSSION

The above data provide us with sufficient information to evaluate the two alternative hypotheses concerning the role of floridoside in red algal cells, namely (1) that floridoside is involved in the restoration of "normal" turgor following a change in external water status (the *osmotic* hypothesis) and (2) that floridoside is simply a photoassimilatory product in red algal cells, having no role in osmotic adjustment (the *non-osmotic* hypothesis). It is clear from Fig. 1(b) that floridoside concentration varied as a direct function of the external salinity, in the manner predicted for an osmotic effector. Since the changes in intracellular water content were not of comparable magnitude, the increases shown in Fig. 1(b) must represent actual enlargements of the internal pool of galactosyl–glycerol and cannot be due to cell shrinkage upon upshock. The *osmotic* hypothesis appears even more favourable when the data for photosynthetic activity are also considered, since this parameter was found to decline in high-salt media. The *non-osmotic* hypothesis would only be supported if floridoside levels declined in parallel with photosynthetic rate upon upshock.

Further indications of the osmotic role of floridoside are contained within Fig. 2. Temporal differences between photosynthetic activity and floridoside concentration suggest that the internal pool of this heteroside does not simply respond to changes in net photosynthesis, since the most rapid increases in intracellular floridoside level occurred during the first hour following transfer to full-strength sea-water, when photosynthetic competence was greatly reduced. Furthermore, floridoside concentration continued to increase up to 24 h after transfer, despite the reduced steady-state level of photosynthetic activity in 100% sea-water [Fig. 2(b)]. These data suggest that floridoside may be formed during the initial stages of upshock from reserve polysaccharide, rather than from newly-fixed carbon compounds. This is in agreement with the observation of Reed *et al.* (1980a) that *P. purpurea* is able to increase its intracellular concentration of floridoside upon upshock in darkness. Comparable data have also been obtained to suggest an osmotically-induced interconversion of polysaccharides and low molecular weight carbohydrates in *Dunaliella* (Müller & Wegmann, 1978) and *Chlorella* (Greenway & Setter, 1979).

The data from ^{14}C-labelling studies are also fully consistent with an osmotic role for floridoside, as this compound showed increasing incorporation of radiocarbon with increasing external salinity (Fig. 3). Since increasing incorporation of ^{14}C into floridoside in high-salt media was also accompanied by a substantial decline in photosynthetic activity (cf. Figs 1 & 3) the case for an *osmotic* (and not a *non-osmotic*) role is strengthened further. The data for *B. atropurpurea* are in accord with the observations of Kauss (1968, 1969) and Kirst & Bisson (1979) regarding the effects of salinity upon ^{14}C incorporation, but

contrast with the findings of Kremer (1979). Kirst & Bisson (1979) have suggested that the apparent contradictions within the published results of these workers may be due to temporal changes in the rates of photosynthetic activity [see Fig. 2(b)], coupled with the fact that Kremer (1979) used short-term incubation in hyposaline and hypersaline media (up to 60 min). The present study has shown that *B. atropurpurea* reaches new steady-state levels of floridoside and rates of photosynthetic activity within 24 h (Fig. 2) and that plants which have been pre-equilibrated for this time period prior to ^{14}C-treatment show osmotically-sensitive variations in ^{14}C-labelling of floridoside [Fig. 3(c)]. It is difficult to imagine that osmotic phenomena are not the primary driving forces behind such responses.

The measured concentration of floridoside in cells of *B. atropurpurea* maintained in sea-water medium is sufficient to balance approximately 13% of the external osmolality (1050 mosmol kg^{-1}), assuming that there is no intracellular compartmentation of this solute. However, should floridoside be localized within a smaller fraction of the cell (e.g. the cytoplasmic compartment, in accordance with the hypothesis that such organic solutes are generally found within the cytoplasm, while inorganic ions are accumulated in vacuoles; see Wyn Jones *et al.*, 1977; Wyn Jones & Gorham, 1983), then the osmotic significance of floridoside would be greatly increased. (It is also worth noting that *B. atropurpurea* collected during the present study appeared to be highly vacuolate upon examination by light microscopy, in contrast to *P. purpurea*, an alga with a small vacuolar component and high floridoside levels; Reed *et al.*, 1980a.)

The low level of floridoside in plant material maintained in freshwater [13·7 mmol kg (cell water)$^{-1}$] is consistent with the requirement for a lower intracellular osmotic pressure to sustain positive turgor under these conditions, since the external osmolality is negligible (measurements of the osmotic potential of canal water gave values less than 20 mosmol kg^{-1}). However, plants growing in a freshwater-based medium will also require an excess of solutes within their cells, by comparison with the external medium and it is thus possible that floridoside also has an osmotic function (albeit at a reduced concentration when compared to cells in sea-water) in cells growing actively in freshwater.

The related compound O-α-D-glucopyranosyl-(1→2)-glycerol (trivial name, lilioside) occurs in salt-stressed cyanobacteria (blue–green algae) and its role in osmotic adjustment has been demonstrated for a number of marine and euryhaline freshwater strains (Borowitzka *et al.*, 1980; Mackay, Norton & Borowitzka, 1983; Richardson, Reed & Stewart, 1983). The similarity between lilioside and floridoside has led Mackay *et al.* (1983) to propose that these cyanobacterial strains may harbour the descendants of floridoside-producing red algal chloroplasts. The functional similarity of these two heterosides may lend some support to this hypothesis, although parallel evolution could also have produced a somewhat similar result.

Kremer & Kirst (1981) have recently shown that the enzymes involved in the biosynthesis of floridoside during photosynthesis are similar in many respects to those described for isofloridoside formation and degradation in *Poterioochromonas* (Kauss, 1978). Further studies are now required to establish the interrelationships between photosynthesis and the formation and degradation of floridoside and floridean starch over a range of salinity, to extend our comprehension of osmotic adjustment in the Rhodophyta.

ACKNOWLEDGEMENTS

This research was supported by an equipment grant (to purchase a Packard 300 liquid scintillation spectrometer) from the Science and Engineering Research Council, UK. The author currently holds a Royal Society Research Fellowship.

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(Accepted 11 March 1985)