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Floridoside, L-Isofloridoside, and D-Isofloridoside in the Red Alga *Porphyra columbina*

Seasonal and Osmotic Effects

Ulf Karsten[1,2]*, Kevin D. Barrow, and Robert J. King

School of Biological Science (U.K., R.J.K.) and School of Biochemistry and Molecular Genetics (K.D.B.), University of New South Wales, Kensington 2033, Australia

The quantitative heteroside distribution in *Porphyra columbina* Montagne and *Bangia atropurpurea* (Roth) C. Agardh (Bangiales, Rhodophyta) has been measured using ^{13}C-nuclear magnetic resonance spectroscopy and gas-liquid chromatography. In *P. columbina*, floridoside and both D- and L-isofloridoside were recorded, with concentrations of L-isofloridoside exceeding those of floridoside. All three compounds were also measured in *B. atropurpurea*. Marked changes in the relative amounts of the heterosides were recorded throughout the season. The role of L-isofloridoside in the osmotic acclimation of *P. columbina* has been demonstrated.

The heteroside floridoside [α-D-galactopyranosyl-(1–2)-glycerol] is considered to be the main photosynthetic and reserve product in all orders of the Rhodophyta except the Ceramiales. Members of the Ceramiales synthesize and accumulate the chemically related digeneaside [α-D-mannopyranosyl-(1–2)-glycerate] (Kirst, 1980). Lindberg (1955) provided evidence that, in addition to floridoside, members of the Bangiales contain an isomeric form of floridoside, isofloridoside [α-D-galactopyranosyl-(1–1)-glycerol]. Wickberg (1958) later reported isofloridoside in *Porphyra umbilicalis* as an isomorphous mixture of D and L forms, but Peat and Rees (1961) found only floridoside and D-isofloridoside in this species. The molecular structures of these closely related compounds are shown in Figure 1.

It was suggested that these striking differences might result from ecological and seasonal factors, or be attributed to taxonomic confusion in the genus *Porphyra* (Peat and Rees, 1961). Recently, the chemical structures and configurations of all heterosides from *Porphyra perforata* were investigated using both NMR and GLC-MS, and the occurrence of floridoside along with both D- and L-isofloridoside was verified (Meng et al., 1987). Although these authors did not publish any quantitative data on the heterosides, they mentioned that the ratio of the D and L forms of isofloridoside in one population of *P. perforata* changed over time: in winter (November), the ratio of D to L form was 1:1; whereas in samples collected in early summer (May), the ratio was 0.01:1. In a recent publication, Meng and Srivastava (1993) reported on diurnal and seasonal variations in floridoside concentration in *P. perforata* from Canada. These authors demonstrated a steady increase in floridoside content from February to May, and also noted some indefinite changes in isofloridoside level, but without any differentiation between the D and L forms.

The biological function of the heterosides as organic compounds involved in osmotic acclimation has been investigated (Kauss, 1967a, 1967b; Reed et al., 1980; Wiencke and Läuchli, 1981; Reed, 1985), but in these studies the D and L forms of isofloridoside were not distinguished, and sometimes even floridoside and isofloridoside were not separately measured. ^{14}C-Incorporation studies on *P. umbilicalis* demonstrate that floridoside is the major photoassimilated compound, and that isofloridoside is very slow in labeling (Craigie et al., 1968). Studies of salinity effects on the concentration of heterosides in Bangiales show that only floridoside plays an important role in the osmotic acclimation. The content of floridoside increases linearly with increasing salt treatment, whereas the amount of isofloridoside (no differentiation between D and L form) remained almost unchanged (Reed et al., 1980; Reed, 1985). It was concluded that within the Bangiales, floridoside is metabolically much more active than isofloridoside.

In the present study of a population of *Porphyra columbina* Montagne from Sydney, Australia, the amounts of floridoside and the D and L form of isofloridoside were quantitatively measured throughout the main growing season by ^{13}C-NMR and GLC techniques. The distribution pattern of these compounds was also determined for several geographically isolated summer populations of *P. columbina* and also in *Bangia atropurpurea* (Roth) C. Agardh. Additionally, the effect of osmotic stress on the concentrations of floridoside and D- and L-isofloridoside was investigated.

MATERIALS AND METHODS

The marine red alga *Porphyra columbina* Montagne was collected from the mid eulittoral zone of the rock platform at

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[2] Present address: University of Bremen, Department of Biology, Marine Botany, 28334 Bremen, Germany.

* Corresponding author; fax 49–421–2184042.

Abbreviations: ppm, parts per million; ppt, parts per thousand.

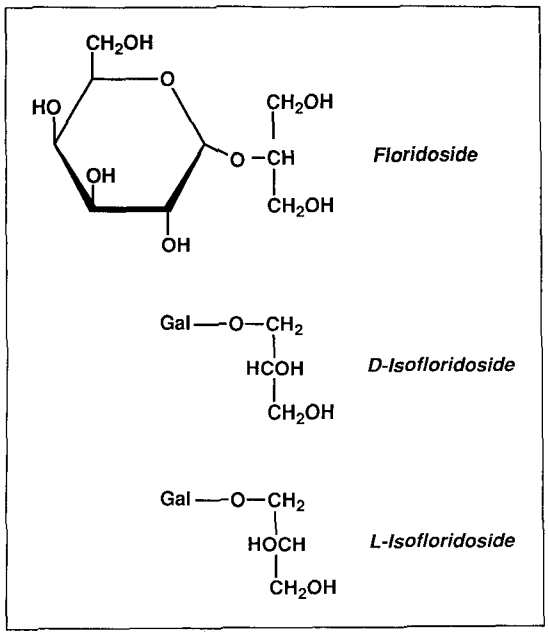

Figure 1. Chemical structures of floridoside [α-D-galactopyranosyl-(1–2)-L-glycerol], L-isofloridoside [α-D-galactopyranosyl-(1–1)-L-glycerol], and D-isofloridoside [α-D-galactopyranosyl-(1–1)-D-glycerol].

Maroubra (Sydney, Australia) (Fig. 2). At this site, macroscopic plants appear in late winter and the population all but disappears by the end of the year. Samples were taken during the entire season from July until December, 1992. In addition, populations along the coastline of New South Wales and from one location in Queensland were collected (Fig. 2) and analyzed for comparative purposes. The closely related *Bangia atropurpurea* (Roth) C. Agardh was isolated from the upper littoral zone at Bronte Beach some 3 km north of Maroubra in July and November, 1992.

After collection, all samples used in analyses were repeatedly blotted with paper tissue to remove extracellular water and then immediately air dried in the laboratory or in the field. For the GLC measurements, the algal samples (10–15 mg dry weight) were extracted in 1 mL of 70% ethanol (v/v) for about 3 h in a water bath at 70°C. These were then centrifuged for 5 min at 5000*g*. Ten microliters of the internal standard *myo*-inositol (1 mg mL^{-1}) were added to 30 to 100 μL of the sample supernatant or to a standard of known concentration and evaporated to dryness under a stream of dry air. The residue was redissolved in 150 μL of pyridine and 20 μL of the silylation reagent *N*-trimethylsilylimidazole (Pierce Chemical Co.), shaken for 30 s, and left overnight. In addition, aliquots of the samples and standards were acetylated by evaporation to dryness followed by adding 200 μL of pyridine and 100 μL of acetic anhydride and heating for 1 h at 70°C. The silylated and acetylated samples were analyzed directly by GLC.

GLC determinations were performed with a Hewlett-Packard 5890A instrument equipped with a flame-ionization detector and connected to an automatic injector 7633. Data were analyzed and processed using a Hewlett-Packard GC ChemStation. A fused-silica capillary column (BP5, 25 m, 0.32 mm i.d.) was used with hydrogen as carrier gas at a head pressure of 10 p.s.i. The heterosides were separated with a temperature program: the oven temperature was kept for 1 min at 150°C following injection (1 μL) and then raised at 40°C min^{-1} to 260°C, where it was left for 2 min. The injection port and the detector were heated to 300°C.

For the ^{13}C-NMR measurements, 150 to 250 mg (dry weight) of algae were extracted in 5 mL of 70% ethanol (v/v) for 3 h in a water bath at 70°C. After centrifugation at 5000*g*, the supernatant was evaporated to dryness and redissolved in 0.5 mL of D_2O (99.98%) for NMR spectroscopy. ^{13}C-NMR spectra were recorded on a Bruker AM-500 spectrometer at 125.77 MHz. Typically, a sweep width of 30,000 Hz, 16,000 time domain points, and a 60° pulse of 3.5 μs were used for acquisition, with composite pulse decoupling. The free induction decay was zero-filled to 32,000 data points and processed with a line broadening of 1.5 Hz. Samples were run at 27°C and referenced from dioxane (67.4 ppm).

Calibration was made using a pure floridoside standard isolated from the red alga *Delisea pulchra* (Greville) Montagne, collected at Cape Banks, Botany Bay, Sydney. The standard was prepared in the following manner. The algal material (1 kg fresh weight), free of epiphytes, was rinsed for 5 min under tap water and then oven-dried overnight at 120°C. The dried tissue was homogenized with a mortar, and 100 g dry weight of the resulting powder was extracted with 500 mL of 70% ethanol (v/v) for 6 h in a water bath at 70°C. The extract was clarified by passing it through several layers of cotton gauze followed by filtration (Whatman GF/C); it was then evaporated to about 100 mL in a rotary evaporator. Pigments and nonpolar compounds were removed by extraction with ethyl acetate (3 × 100 mL) in a separation funnel. The lower water phase was then desalted by passing it through columns of AG 50WX2 (H^+ form) and AG 1X8 (OH^- form) ion-exchange resins (Bio-Rad Laboratories). The neutral eluent was evaporated to dryness and redissolved in hot

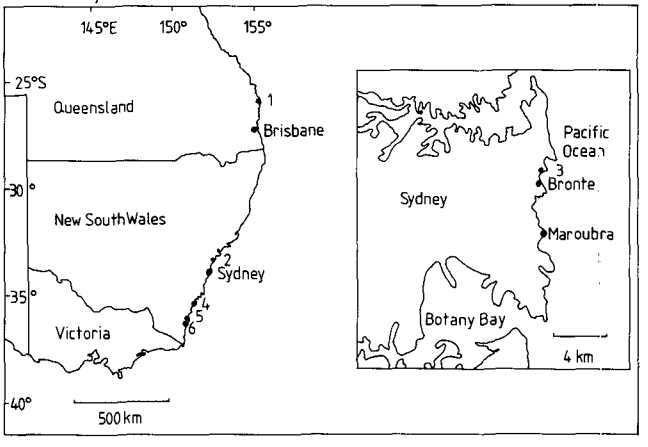

Figure 2. Map of the collection locations for the different populations of *P. columbina* from Queensland and New South Wales, Australia, and for the populations of *P. columbina* and *B. atropurpurea* from the Sydney region used for the investigation of seasonal effects.

methanol to remove the insoluble oligo- and polysaccharides. After evaporation to dryness, the residue was redissolved in double-distilled water and passed through a column of Dowex 50 (Ca^{2+} form). The resulting eluent was again evaporated to dryness and redissolved in hot methanol. Floridoside was crystallized from the methanol fraction after partial evaporation in a rotary evaporator, and the purity was checked by NMR and GLC techniques. The standard for D- and L-isofloridoside was kindly supplied by Dr. J.S. Craigie (National Research Council, Halifax, Canada) as a 1:1 (w/w) mixture.

The salinity experiments were conducted with young plants in September, 1992. Freshly collected material of *P. columbina* from Maroubra was cleaned of the few epiphytes present and briefly rinsed with tap water. Plants were then incubated for 72 h in 500-mL Pyrex dishes at a range of different salinities (10, 34, and 60 ppt). Diluted seawater was obtained by mixing natural seawater with glass-distilled water, and concentrated seawater was prepared by adding artificial sea salt (SEA-SALT, Sigma, Ltd.). All media were enriched with PES/2 (Starr and Zeikus, 1987) and 3 to 4 mM $NaHCO_3$. The algae were maintained in a growth cabinet at 25°C, with 30 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent tubes and a light:dark cycle of 16:8 h. Afterward, plants were repeatedly blotted with paper tissue and oven dried for 24 h at 60°C.

RESULTS

The ^{13}C-NMR chemical shifts of floridoside and L- and D-isofloridoside are summarized in Table I. The anomeric signals of the C-1 atom at 98.9, 99.3, and 99.6 ppm clearly show that three heterosides are present. The single signal at 79.6 ppm belongs to the C-2′ atom from the glycerol residue of floridoside and is a characteristic marker for this compound. The seasonal changes in the intracellular concentrations of the heterosides floridoside, L-isofloridoside, and D-isofloridoside in *P. columbina* and *B. atropurpurea* are shown in Figures 3 and 4.

P. columbina is normally present at the collection site in Maroubra, Sydney (Fig. 2), for about 6 to 7 months from June/July until December/January. In Australian winter (July), young plants of *P. columbina* exhibited nearly equimolar and low concentrations of floridoside and L-isofloridoside (130 and 160 mmol kg^{-1} dry weight, respectively), whereas only traces of D-isofloridoside (2 mmol kg^{-1} dry weight) could be detected (Fig. 3). Between July and the beginning of November, the floridoside level remained unchanged, but it increased 2.3-fold toward the end of November. A slight decrease of the floridoside content by about 25%, however, was recorded at the beginning of December. This value remained nearly unaffected during all of December until the disappearance of the population.

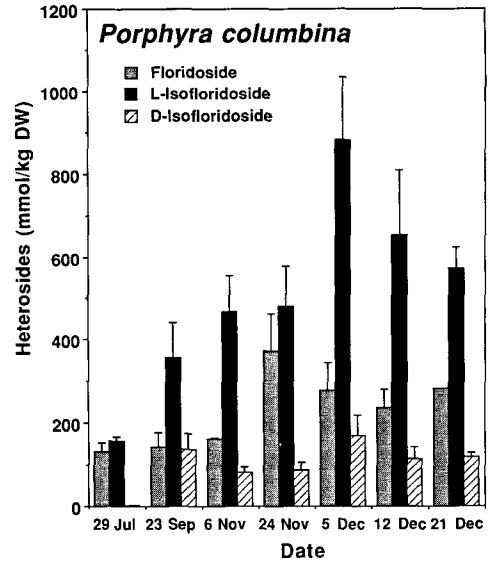

Figure 3. Seasonally changing concentrations of floridoside, L-isofloridoside, and D-isofloridoside in a natural epilithic population of *P. columbina* from the mid eulittoral zone of the rock platform at Maroubra (Sydney, Australia). Data are expressed as mean value ± SD (n = 4).

In contrast to the unchanged floridoside content between July and early November, L-isofloridoside was continuously rising over this period to a concentration of 470 mmol kg^{-1} dry weight (Fig. 3). Between early and late November, however, when the floridoside level strongly increased, the L-isofloridoside value remained unchanged. This period was followed by a marked increase in L-isofloridoside at the beginning of December, peaking at a maximum content of about 900 mmol kg^{-1} dry weight. During the middle and end of December, however, the L-isofloridoside value linearly decreased by about 35%.

The proportionally largest increase in heteroside concentration (70-fold) was observed for D-isofloridoside in young plants between July and September, after which it declined, from 135 mmol kg^{-1} dry weight in September to 85 mmol kg^{-1} dry weight in early November. Throughout November, the content remained essentially unchanged, but at the beginning of December it doubled, and a maximum value of 170 mmol kg^{-1} dry weight was found. This concentration gradually decreased through the end of the season to 120 mmol kg^{-1} dry weight (Fig. 3).

Table I. *^{13}C-NMR chemical shifts of the heterosides*

^{13}C-NMR chemical shifts of floridoside, L-isofloridoside, and D-isofloridoside in 99.98% D_2O referenced from internal dioxane at 67.4 ppm. C-1 to C-6 belong to Gal, and C-1′ to C-3′ to glycerol.

Carbon	Chemical Shifts		
	Floridoside	L-Isofloridoside	D-Isofloridoside
		ppm	
C-1	98.9	99.3	99.6
C-2	69.3	69.5	69.3
C-3	70.2	70.3	70.3
C-4	70.1	70.1	70.1
C-5	71.9	71.8	71.8
C-6	62.3	62.0	62.0
C-1′	61.9	71.5	71.3
C-2′	79.6	69.5	69.8
C-3′	61.3	63.4	63.4

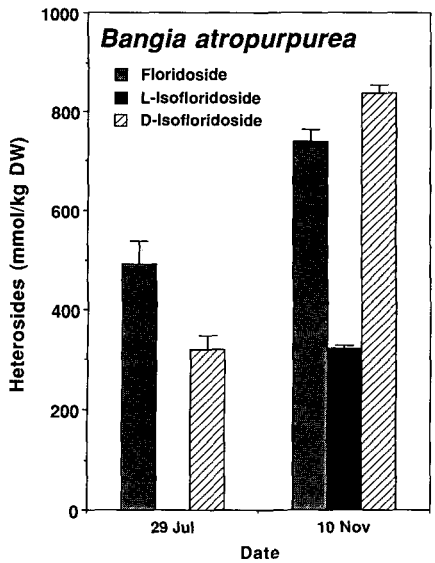

Figure 4. Seasonally changing concentrations of floridoside, L-isofloridoside, and D-isofloridoside in a natural epilithic population of *B. atropurpurea* from the upper eulittoral zone at Bronte Beach (Sydney, Australia). Data are expressed as mean value ± SD ($n = 4$).

In contrast to *P. columbina*, *B. atropurpurea* in the Sydney region is conspicuous for a much shorter period of only 3 to 4 months. In young plants collected in July, there was 490 mmol kg^{-1} dry weight floridoside and 320 mmol kg^{-1} dry weight D-isofloridoside, but no traces of L-isofloridoside were detected (Fig. 4). At the end of the season in November, however, high L-isofloridoside concentrations were recorded (320 mmol kg^{-1} dry weight). Whereas the floridoside value rose during this period by more than 50%, the D-isofloridoside content showed a proportionally even stronger increase of more than 160%, peaking in a maximum concentration of 840 mmol kg^{-1} dry weight.

The heteroside distribution patterns in geographically different summer populations of *P. columbina* are presented in Table II. All isolates from the coastline of New South Wales had a very similar distribution pattern, with very low concentrations of D-isofloridoside ranging from 55 to 148 mmol kg^{-1} dry weight. Although the content of floridoside was in the midrange (230–445 mmol kg^{-1} dry weight), all populations exhibited highest values for L-isofloridoside from 481 to 736 mmol kg^{-1} dry weight (Table II). The isolate from Queensland, however, had a very low floridoside level and nearly equimolar and high concentrations of L- and D-isofloridoside. In all populations of *P. columbina*, the three heterosides amounted to between 20.5 and 33.8% of the dry weight.

Typical ^{13}C-NMR spectra were obtained consistently throughout the study, and examples are shown in Figure 5. The effect of salinity on the intracellular floridoside and L- and D-isofloridoside concentrations of *P. columbina* from Maroubra, Sydney, is also shown in Figure 5, as well as in Figure 6. The ^{13}C-NMR spectra and the GLC measurements show that L-isofloridoside is the quantitatively predominant heteroside in all treatments and that its content increased linearly with increasing salinities. The floridoside concentration was highest in normal seawater conditions (34 ppt), with a marked decrease in both hyposaline (10 ppt) and hypersaline (60 ppt) conditions. The D-isofloridoside content remained unchanged between 10 and 34 ppt, but increased by a factor of 2.7 in plants kept at 60 ppt (Fig. 6).

DISCUSSION

NMR spectroscopy is now a well-established technique for studying organic solutes that may be involved in osmotic acclimation (Reed et al., 1985). All major organic solutes, including all classes of compounds, may be detected and identified in cell extracts. It is possible to obtain accurate quantitative information from ^{13}C-NMR spectra, but this was not done in the current study. To obtain quantitative results, relaxation data and nuclear Overhauser effects must be measured and taken into account, or a series of standards must be added to each sample. The spectra shown in Figure 5 and all the ^{13}C-NMR spectra in this work were run under partially relaxed conditions, using a 60° pulse and a pulse repetition rate of 2 s. Under these conditions, carbons in the same or very similar environment (i.e. showing identical T_1 relaxation times and nuclear Overhauser effects) will give quantitative information, and, for example, the signals for the anomeric carbons at 98 to 99 ppm can be compared directly. However,

Table II. *Heteroside concentration in different P. columbina populations*

The concentrations of the heterosides floridoside, L-isofloridoside, and D-isofloridoside in different populations of *P. columbina* from the coastline of New South Wales (NSW) and from one location in Queensland (Qld), Australia. The numbers of the locations refer to those shown in Figure 2. The contents are given as mean value ± SD ($n = 3$–4) and expressed as mmol kg^{-1} dry weight. The sum of all three heterosides is also presented as percentage of the dry weight.

Location and Date	Floridoside	L-Isofloridoside	D-Isofloridoside	Total (% dry wt)
Noosa Head, Qld, 29 Dec 92	132.8 ± 33.7	591.8 ± 76.9	538.9 ± 70.1	32.1 ± 4.6
Terrigal, NSW, 13 Jan 93	285.1 ± 24.0	641.5 ± 96.1	108.9 ± 16.3	26.3 ± 3.5
Tamarama, NSW, 24 Nov 92	445.2 ± 53.4	515.7 ± 46.6	55.2 ± 5.6	25.8 ± 2.7
Tamarama, NSW, 5 Dec 29	241.7 ± 26.2	697.8 ± 100.3	134.0 ± 19.3	33.8 ± 3.7
Ulladulla, NSW, 11 Jan 93	255.0 ± 121.8	481.6 ± 242.7	71.4 ± 36.0	20.5 ± 10.2
Narooma, NSW, 12 Jan 93	282.1 ± 70.5	736.3 ± 67.9	131.5 ± 12.1	29.2 ± 3.8
Bermagui, NSW, 13 Jan 93	230.8 ± 42.1	621.5 ± 206.4	148.3 ± 49.2	25.4 ± 7.6

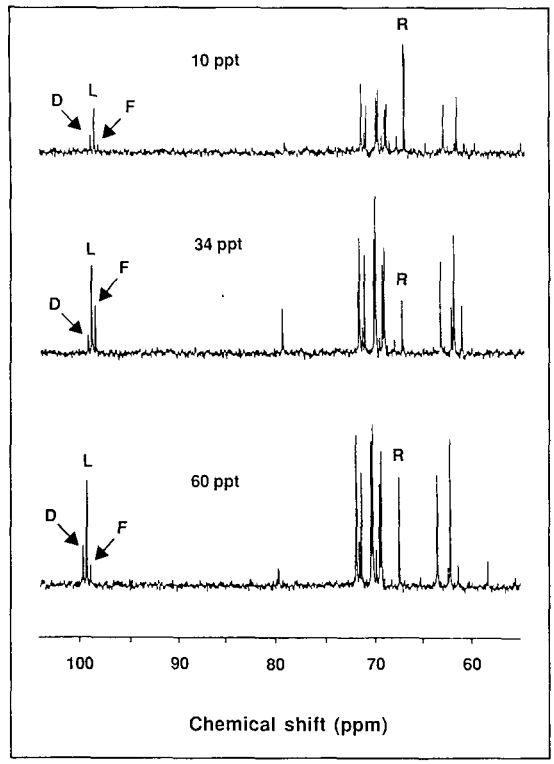

Figure 5. ^{13}C-NMR spectra of the 70% ethanol extracts of *P. columbina* treated for 72 h with salinities of 10, 34, and 60 ppt. For each extraction, exactly the same dry weight (150 mg) was utilized. R is the resonance for the internal standard dioxane. D, L, and F refer to the anomeric carbons of D-isofloridoside, L-isofloridoside, and floridoside, respectively.

the NMR method is rather insensitive, very time consuming, and needs a considerable amount of sample. We prefer to use the NMR method to confirm the composition of low mol wt organic compounds present and then use a more sensitive detection method, in this case capillary GLC with an autoinjector, so that many more determinations can be carried out, which are statistically more reliable.

The results shown in Figures 3 and 4 indicate a strong seasonal effect on the quantitative and qualitative heteroside distribution pattern in *P. columbina* and *B. atropurpurea*. A similar seasonal variation in the floridoside concentration was described for *P. perforata* from the Northern hemisphere (Meng and Srivastava, 1993). Such seasonal responses are generally related to environmental light and temperature regime. In warm temperate/temperate regions such as Sydney, however, it is related mainly to seasonally fluctuating daylengths and, to a minor extent, to changing light intensities and temperatures during the course of the year. The influence of a seasonally fluctuating light factor on macroalgal photosynthesis (King and Schramm, 1976; Gutkowski and Maleszewski, 1989), development (Wiencke, 1990), and the concentration of the sulphonium compound β-dimethylsulphoniopropionate (Karsten et al., 1990), which acts as an organic osmolyte (Karsten et al., 1991), is well documented: LD conditions, for example, lead to a strong accumulation of β-dimethylsulphoniopropionate in cells of green macroalgae. It can be hypothesized that the seasonally increasing heteroside concentrations in *P. columbina* and *B. atropurpurea* are light dependent and a response to increasing daylength. The underlying biochemical mechanisms of these phenomena are still unknown, but the hypothesis of light stimulation of enzymes involved in heteroside biosynthesis is strengthened by reports on light-activated enzymes of the amino acid metabolism in microalgae (Tischner and Hüttermann, 1980). The activity of the key enzyme for floridoside biosynthesis, floridoside phosphate synthase, is known to be related to the season (Meng and Srivastava, 1993). These authors reported on a steady and strong increase in activity from February to April, i.e. with increasing daylengths, and their data, thus, support the idea of light-stimulated enzymes.

The quantitatively very different composition of the contents of floridoside, L-isofloridoside, and D-isofloridoside in *P. columbina* for each sampling date points to different and/or variable enzyme activities for the synthesis of the three heterosides. This hypothesis is supported by a study that demonstrates strong seasonal variation in the activity of a whole set of enzymes extracted from the brown alga *Laminaria hyperborea* (Küppers and Weidner, 1980). Although the metabolic pathways for the biosynthesis of floridoside in marine Rhodophyceae (Kremer and Kirst, 1981) and of L-isofloridoside in the freshwater Chrysophyte *Poterioochromas malhamensis* (= *Ochromonas malhamensis*) (Kauss, 1977) are experimentally verified, that for D-isofloridoside is still unknown. In the case of floridoside in red algae and L-isofloridoside in *P. malhamensis*, the biosynthesis is initiated by a condensation of L-glycerol-3-P and UDP-galactose to floridoside-P and L-isofloridoside-P, respectively. The latter compounds are cleaved to the corresponding heteroside by de-

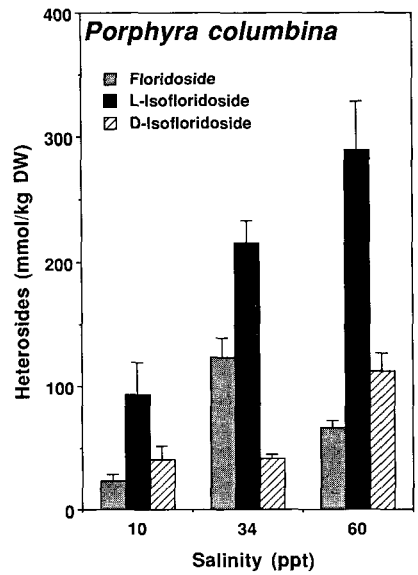

Figure 6. The effect of salinity on the intracellular concentrations of floridoside, L-isofloridoside, and D-isofloridoside in *P. columbina*. Plants were collected in September, 1992, and treated for 72 h with salinities of 10, 34, and 60 ppt. Data are expressed as mean value ± SD (n = 3).

phosphorylation. Although for the biosynthesis of floridoside and L-isofloridoside, L-glycerol-P is a precursor, the only difference between the molecules is the location of the condensation reaction, which takes place at the C-2 or C-1 position of glycerol (Fig. 1).

Meng and Srivastava (1991), however, reported on a high substrate specificity of the floridoside phosphate synthase isolated from *P. perforata*. Their partially purified enzyme formed only floridoside and not even traces of isofloridoside. Thus, these authors concluded that isofloridoside is synthesized by a different enzyme using different substrates, or by the isomerization of floridoside. Due to the fact that during the seasonal investigation the D-isofloridoside concentration of *P. columbina* did not follow that of L-isofloridoside, another independent metabolic pathway for this heteroside can be assumed. The simplest explanation for the biosynthesis of D-isofloridoside may involve a D-glycerol derivative as a precursor analogous to the L-isofloridoside synthesis. It is interesting that the osmoregulatory response in *Escherichia coli* involves phosphatidyl glycerol as the source of the phosphoglycerol substituents on the membrane-derived oligosaccharides (Jackson and Kennedy, 1983; Jackson et al., 1984). These oligosaccharides contain 10 to 12 residues linked β-1,2 and β-1,6, and are substituted at the 6-position by *sn*-1-glycerol-P (this corresponds in stereochemistry to the D-glycerol nomenclature used in describing the floridosides). Phosphatidyl glycerol is the only well-established source of the *sn*-1-glycerol-P (D-glycerol) in intermediary metabolism and may be the source of these units in D-isofloridoside biosynthesis. Detailed enzymological work on this is required for the Bangiales.

Figures 5 and 6 clearly demonstrate L-isofloridoside to be the predominant heteroside involved in the osmotic acclimation of *P. columbina*, since the concentration significantly and proportionally changes with the salinity in the culture conditions. Although D-isofloridoside played only a minor role as osmolyte under hypersaline conditions, the floridoside content strongly decreased after hypo- as well as hyperosmotic treatments. These data are in sharp contrast to other studies on the osmotic acclimation in *Porphyra* species.

The function of the heterosides as organic osmolytes in salt-treated red algae was first proposed by Kauss (1968). Using ^{14}C-label, he showed that floridoside in *P. perforata* increased markedly with increasing salinities over the range 50 to 200% seawater. Although Kauss (1968) did not distinguish between L- and D-isofloridoside, he first demonstrated the accumulation of isofloridoside, but only at very low salt concentrations between 50 and 100% seawater; further increase in salinity up to 200% seawater was accompanied by a decline of the isofloridoside concentration. The role of floridoside as an important osmoticum in the cytoplasm has been proven for many different red algae from a diversity of orders in the Florideophycideae (Kirst and Bisson, 1979), and also in the Bangiales (*Porphyra purpurea* [Reed et al., 1980], *Porphyra umbilicalis* [Wiencke and Läuchli, 1981], and *B. atropurpurea* [Reed, 1985]). Whereas Wiencke and Läuchli (1981) determined only the total heteroside content between 50 and 300% seawater in their studies on *Porphyra*, Reed et al. (1980) distinguished between floridoside and isofloridoside, although not between the L and D form of the latter. Reed et al. (1980) showed that although isofloridoside was present in relatively high concentrations in *P. purpurea*, only floridoside varied in response to external salinity. On a daily basis, however, salinity did not influence the floridoside level of *P. perforata* (Meng and Srivastava, 1993). The closely related *B. atropurpurea* synthesized and accumulated high contents of floridoside in hypersaline conditions, while isofloridoside remained a minor compound (Reed, 1985). To our knowledge, an important role of isofloridoside as an organic osmolyte has been described only for *P. malhamensis*, a unicellular flagellate that lacks the ability to synthesize floridoside (Kauss, 1967a, 1967b).

This study is the first to quantify all naturally occurring heterosides in one species of *Porphyra* and to record the function of L-isofloridoside, and to a lesser extent of D-isofloridoside, as major organic osmolytes in algae under hypersaline stress. The differences between the data of Kauss (1968), Reed et al. (1980), and Wiencke and Läuchli (1981) and our results may be related to the different *Porphyra* species being studied, as well as to the advanced analytical techniques utilized in the present investigation.

The salt experiments were done with young plants in early September, 1992, and therefore the heteroside concentrations are relatively low at 60 ppt (Fig. 6). It may be assumed that the overall contents of these compounds in *P. columbina* are much higher when they are hypersaline and treated later in the season. Besides their role in osmotic acclimation, the heterosides may also serve as soluble carbon reserves. Due to the fact that an organic compound cannot act simultaneously as carbon source and as osmolyte (unless it is replaced by a solute of equal utility) (Raven et al., 1990), it seems that these physiological functions are shared among the three heterosides. Although L-isofloridoside is the main osmolyte, floridoside may function more as a carbon storage compound.

The presence, synthesis, and accumulation of three isomeric heterosides raises the question of biological function. Heterosides and other low mol wt organic compounds such as polyols, Pro, Gly, and betaine are thought to function as compatible solutes having stabilizing effects on enzymes, membranes, and structural macromolecules against inhibition, inactivation, and denaturation under conditions of low water potential (Kirst, 1990). Such physiological functions of the heterosides would be essential for survival of *Porphyra* and *Bangia*, which normally grow in the upper and mid eulittoral zones, where plants experience extremes of abiotic factors. The presence of one heteroside would, however, be sufficient for all physiological requirements of a compatible solute. Although the presence of more than one heteroside involves different enzyme systems and hence is expensive in terms of metabolic energy, no obvious hypothesis suggests itself. The limited data thus far suggest that there may be some taxonomic basis for the phenomenon. The order Bangiales, including the genera *Bangia* and *Porphyra*, forms a well-accepted and monophyletic group based on life history and developmental features and is classified as primitive relative to other red algal taxa (Garbary and Gabrielson, 1990). All other red algal orders, except the Ceramiales, synthesize and accumulate only one heteroside (floridoside), which, thus, seems to be a derived character.

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