Planta (2005) 222: 319–326
DOI 10.1007/s00425-005-1527-3

ORIGINAL ARTICLE

Ulf Karsten · Dirk Michalik
Manfred Michalik · John A. West

A new unusual low molecular weight carbohydrate in the red algal genus *Hypoglossum* (Delesseriaceae, Ceramiales) and its possible function as an osmolyte

Received: 20 December 2004 / Accepted: 21 February 2005 / Published online: 22 April 2005
© Springer-Verlag 2005

Abstract The low molecular weight carbohydrates in various species of the red algal genus *Hypoglossum* (Delesseriaceae, Ceramiales) were analysed using HPLC, ^{1}H and ^{13}C-nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. All specimens contained the heteroside digeneaside which is considered as chemosystematic marker for the Ceramiales. A new HPLC method was developed for the separation and quantification of this compound, and concentrations between 131.6 mmol kg^{-1} and 539.6 mmol kg^{-1} DW could be measured among the species tested. In addition, during the HPLC analysis another new low molecular weight carbohydrate was detected in two species from The Philippines (*H. barbatum*) and Western Australia (*H. heterocystideum*), and its chemical structure elucidated as digalactosylglycerol applying various NMR experiments. The remaining *Hypoglossum* taxa lack this compound. Although digalactosylglycerol occurred in high concentrations in the range of 221.7 and 438.7 mmol kg^{-1} DW in *H. barbatum* and *H . heterocystideum*, respectively, it has never been reported for any other algal species before. Therefore, to test the possible physiological function of this unusual carbohydrate as organic osmolyte, *H. barbatum* was treated with a range of salinities. While the digeneaside content remained almost unchanged, the digalactosylglycerol concentration strongly increased with increasing salinities from 70 mmol kg^{-1} DW at 20 psu to 215 mmol kg^{-1} DW at 45 psu. In conclusion, while neither published work nor the present study indicate digeneaside to play more than a minor role in osmotic acclimation, the data presented strongly support an osmotic function of digalactosylglyerol.

Keywords Digeneaside · Digalactosylglycerol · Organic osmolyte · Osmotic acclimation · Rhodophyta · Salt tolerance

Abbreviation (*psu*): practical salinity units

U. Karsten (✉)
Institute of Biological Sciences,
Applied Ecology, University of Rostock,
Albert-Einstein-Str. 3, 18051 Rostock, Germany
E-mail: ulf.karsten@biologie.uni-rostock.de
Tel.: +49-381-4986090
Fax: +49-381-4986072

D. Michalik · M. Michalik
Leibniz Institute for Organic Catalysis,
University of Rostock, 18055 Rostock, Germany

J. A. West
Department of Botany, University of Melbourne,
Parkville, 3052, VIC, Australia

Introduction

The main photosynthetic and reserve product in members of most orders of Rhodophyta is the heteroside floridoside (α-D-galactopyranosyl-(1-2)-glycerol). In contrast, Ceramialean taxa generally synthesize and accumulate instead of floridoside the chemically related digeneaside (α-D-mannopyranosyl-(1-2)-glycerate Kremer 1978). Kremer and Vogl (1975) proposed the presence of this compound as a chemosystematic marker for the Ceramiales. In addition, all members of this order are not only uniaxial, but are also separated from all other red algal orders on the basis of post-fertilization events in the female gametophyte, that is, the auxiliary cells are formed after rather than before fertilization (Garbary and Gabrielson 1990). Molecular data on the *rbcL* gene also support the supposed monophyletic position of the Ceramiales (Freshwater et al. 1994).

More recent studies, however, indicate a more complex picture on the diversity and distribution of low molecular weight carbohydrates among the Rhodophyta. Members of the Bangiophycean order Bangiales such as *Porphyra* and *Bangia* contain in addition to floridoside two isomeric forms of floridoside, D-isofloridoside (α-D-galactopyranosyl-(1-1)-D-glycerol) and L-isofloridoside (α-D-galactopyranosyl-(1-1)-L-glycerol Meng et al. 1987; Karsten et al. 1993). Although the

distribution of different heterosides within red algal orders had been regarded as chemotaxonomically useful (Kremer and Vogl 1975; Kremer 1978), recently it has been demonstrated that some genera of the Ceramiales, *Laurencia* and *Osmundea*, do in fact produce and accumulate floridoside and not digeneaside (Barrow et al. 1995). Other biochemical exceptions in terms of major carbohydrates are also recorded in this order, with taxa such as *Bostrychia*, *Stictosiphonia* and *Caloglossa* characterized by polyols such as sorbitol, dulcitol and mannitol compounds which are otherwise very unusual in the red algae (Karsten et al. 1992a, 1992b). Most interesting is the fact that all known low molecular weight carbohydrates found in the Ceramiales, that is, heterosides and polyols, are evident in the Bangiophyceae as well (Karsten et al. 1999, 2003). Since the bangiophycean orders are considered to be more ancestral relative to the Florideophyceae (Pueschel 1987; Garbary and Gabrielson 1990), the ability to synthesize all of the same compounds found in the 'advanced' Florideophyceae indicate the maintenance of ancestral physiology in advanced taxa.

Because of these exceptional biochemical features ^{13}C-nuclear magnetic resonance spectroscopy (^{13}C NMR) was used to screen for further new low molecular weight carbohydrate patterns among the Ceramiales. The consistent presence of digeneaside together with a new compound was noted in some members of the genus *Hypoglossum* (Delesseriaceae, Ceramiales), and hence a survey in members of this taxon was undertaken. A new HPLC method for the quantification of digeneaside was developed and the chemical structure of the unknown carbohydrate elucidated. To provide first information on the possible function of this new low molecular weight carbohydrate as an organic osmolyte, algal samples were treated with a range of salinities.

Materials and methods

Plant material

Collection locations and dates of the species used for the low molecular weight carbohydrate analysis are presented in Table 1. All algae were grown as unialgal cultures in sterile seawater (30–32 practical salinity units (psu)) enriched with Modified PES/2 (West and McBride 1999). Cultures were maintained in 500-ml Pyrex dishes at 23–25°C in a climatic chamber and irradiated with 20–30 μmol photons $m^{-2}s^{-1}$ using cool-white fluorescent tubes under a photoperiod of 16 h light: 8 h darkness. The algae were grown in continuous immersion culture; the medium was changed every 2 weeks. All algal samples were oven-dried at 40°C overnight prior to chemical analysis. This treatment did not affect the low molecular weight carbohydrate concentrations, and such samples could be stored for many months without any degradation when kept under dry, cool and dark conditions.

Digeneaside extraction and crystallization

Digeneaside was isolated from the red alga *Ceramium rubrum* (Hudson) C. Agardh collected from the upper sublitoral of the rocky island Helgoland (North Sea, Germany). About 50 g dried plant material were extracted with 500 ml of 70% ethanol (v/v) for 3 h in a waterbath at 80°C, followed by filtration over several layers of Miracloth and evaporation to dryness under reduced pressure. Pigments and nonpolar compounds were removed by treatment with trichloromethane, the residue dissolved in water and then applied to a self-packed 100-ml column of Amberlite XAD-4 (Sigma) to eliminate further hydrophobic contaminations. The aqueous eluant was again evaporated to dryness under reduced pressure, dissolved in 10-ml 10% methanol (v/v) and applied to a commercial Sep-Pack VAC C^{18} -column (Waters) to remove further hydrophobic compounds. The methanolic eluant was evaporated to dryness under reduced pressure, dissolved in 20-ml distilled water and transferred to a self-packed 200 ml column of anion-exchange resin DOWEX 2×8 (OH^- form Sigma). Elution with a linear salt gradient (0–0.3 M NaCl) removed weakly bonded contaminating anions, followed by the application of 100 ml 1 M NaCl to elute digeneaside from the column. The aqueous eluant was again evaporated to dryness under reduced pressure, dissolved in 10 ml distilled water and finally applied to a self-packed 140 ml column filled with a size-exclusion gel Superdex 30 prep grade (Pharmacia Biotech). The digeneaside containing aqueous fractions were combined, evaporated to a final volume of about 2 ml under reduced pressure, and mixed with 50 ml ice-cold 100% ethanol (v/v) until small white crystals were visible. The Na^+ -digeneaside crystals were harvested by centrifugation (10,000 g), dried at 60°C overnight (253 mg) and stored frozen at −20°C. The crystals exhibited a melting point of 190–192°C, which is identical with the data of Barrow et al. (1993).

Digeneaside and low molecular weight carbohydrate quantification

Digeneaside and other low molecular weight carbohydrates were separated and quantified by HPLC. For these analyses, the algal samples (10–15 mg DW) were extracted in 1 ml of 70% (v/v) ethanol for about 3 h in a waterbath at 70°C. After centrifugation for 5 min at 5,000 g, 700 μl of the supernatant was evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were re-dissolved in 700 μl distilled water and vortexed for 30 s. After passing through a 0.2 μm membrane filter, samples were analysed with an isocratic Agilent HPLC system equipped with a differential refractometer. Carbohydrates were separated on a stainless-steel Phenomenex Rezex ROA-Organic Acid column (300 × 7.8 mm O.D.) protected with a Phenomenex Carbo-H^+ guard cartridge (4 × 3 mm

Table 1 Origin, digeneaside and digalactosylglycerol concentration in different isolates and species of the genus *Hypoglossum*

Species	Culture no.	Origin	Digeneaside (mmol kg^{-1} DW)	Digalactosylglycerol (mmol kg^{-1} DW)	Total (mmol kg^{-1} DW)
Hypoglossum sp.	2403	Puerto Morales, Quintana Roo, Mexico, 27.7.1980	296.1 ± 37.1	n.t.	296.1
Hypoglossum barbatum Okamura	2926	Talisoy, Virac, Catanduanes, The Philippines, 14.5.1988	177.0 ± 13.4	221.7 ± 45.1	398.7
Hypoglossum tenuifolium (Harvey) J. Agardh	3386	Media Luna Reef, La Parguera, Puerto Rico, 28.11.1985	385.4 ± 58.3	n.t.	385.4
Hypoglossum rhizophorum Ballantine et Wynne	3388	La Parguera, Puerto Rico, 4.12.1985	131.4 ± 9.9	n.t.	131.4
Hypoglossum caloglossoides Wynne et Kraft	3497	Cooks Bay, Moorea, French Polynesia, 3.7.1995	131.6 ± 9.7	n.t.	131.6
Hypoglossum caloglossoides	3499	Cooks Bay, Moorea, French Polynesia, 3.7.1995	194.6 ± 35.7	n.t.	194.6
Hypoglossum caloglossoides	3500	Cooks Bay, Moorea, French Polynesia, 6.7.1995	107.6 ± 2.4	n.t.	107.6
Hypoglossum caloglossoides	3502	Cooks Bay, Moorea, French Polynesia, 4.7.1995	176.2 ± 3.1	n.t.	176.2
Hypoglossum caloglossoides	3503	Cooks Bay, Moorea, French Polynesia, 4.7.1995	156.0 ± 13.9	n.t.	156.0
Hypoglossum heterocystideum (J. Agardh) J. Agardh	3985	Aquarium John Huisman, Murdoch University, Perth, Australia 9.7.1999	539.6 ± 46.8	438.7 ± 51.7	978.3
Hypoglossum hypoglossoides (Stackhouse) Collins& Hervey	4500	Rapada Island, Ubatuba, Brazil, April 1999	304.2 ± 25.1	n.t.	304.2

The concentrations given represent the mean value (± SD) of at least three replicates and are expressed in mmol kg^{-1} DW
n.t. no trace

I.D.). The mobile phase was 5 mM H_2SO_4 run isocratically at a flow rate of 0.5 ml min^{-1} and a temperature of 65°C. Low molecular weight carbohydrates were identified by comparison of retention times with those of standard compounds, and quantified by peak areas. The concentrations are expressed as mmol kg^{-1} dry weight (DW), and represent the mean value ± SD of three replicate measurements.

NMR and MS measurements

For NMR spectroscopy 500 mg of algal DW of *H. barbatum* (2926) was extracted in 5 ml of aqueous ethanol (70% v/v) for 3 h in a waterbath at 70°C. After centrifugation at 5000g, the supernatant was evaporated to dryness in vacuo and redissolved in 0.5 ml of D_2O (99.98%) for NMR spectroscopy. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVANCE 500 spectrometer. (^{1}H: 500.13 MHz; ^{13}C: 125.7 MHz). Chemical shift values δ (in ppm) are given relative to the signal for internal TMS (δ = 0). The calibration of spectra was carried out using the signals of added dioxane (δ (^{1}H) = 3.71; δ (^{13}C) = 67.6). The values for coupling constants J are given in Hz. Typical ^{1}H and ^{13}C NMR spectra of the *Hypoglossum* extract with the signals of anomeric protons and carbon atoms are displayed in Fig. 1 a,b.

The high-resolution positive ion ESI mass spectrum was obtained using a Bruker Daltronics APEX III FT-ICR MS equipped with a 7.0 tesla shielded superconducting magnet and an external electrospray ion source.

Salinity experiment

For the salt stress experiments, *H. barbatum* (2926) was treated for 72 h in 500 ml Pyrex dishes with hypo- (15, 20, 25 psu) and hypersaline (45, 50, 60 psu) media at 25°C, 25–30 µmol photons m^{-2} s^{-1} provided by cool-white fluorescent tubes and a photoperiod of 16 h light : 8 h dark. All osmolalities were prepared from North Sea water (33 psu). While hypersaline media were obtained by adding artificial sea-salt (WIMEX, Wiegand Ltd,

Germany), hyposaline seawater was prepared by mixing with distilled water. Salinity was checked using a refractometer. All experimental media were enriched with 2 mM $NaHCO_3$ to ensure sufficient inorganic carbon supply over the course of treatments. Twice a day the Pyrex dishes were carefully stirred to decrease any gradients in nutrient supply. After 72 h algal samples were harvested, blotted dry using several layers of paper tissue and oven-dried at 45°C overnight. This material was kept under dry, cool and dark conditions prior extraction. Four replicate samples were used for each salinity.

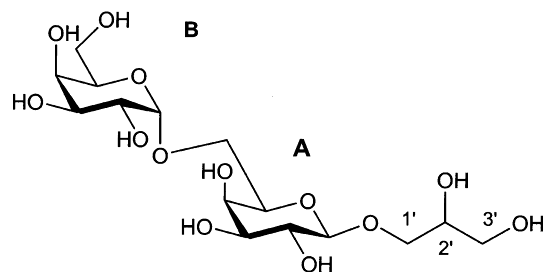

Fig. 2 Chemical structure of the digalactosylglycerol (2, 3-dihydroxypropyl (α-D-galactopyranosyl)-(1 → 6)-β-D- galactopyranoside) in *H. barbatum* (2926)

Results

All specimens of *Hypoglossum* were investigated by ^{13}C-NMR spectroscopy for the qualitative occurrence of low molecular weight carbohydrates (data not shown), and in most strains only digeneaside was present. However, *H. barbatum* (2926) and *H. heterocystideum* (3985) showed in addition to digeneaside strong resonances of another compound.

^{1}H and ^{13}C NMR spectra of *H. barbatum* (2926) in D_2O solution showed the presence of three different compounds: digeneaside, which has been described in detail earlier (Karsten et al. 1994), a disaccharide component and an unknown compound which is oligomeric. Digeneaside and the disaccharide component were found in a molar ratio of about 1:1. The presence of these compounds can easily be detected by the signals in the anomeric region covering both the ^{1}H and ^{13}C NMR spectra (Fig. 1 a,b).

From the dried extract the disaccharide component could be isolated by column chromatography via the peracetylated derivative and followed by deacetylation. By means of one- and two-dimensional NMR spectroscopy the structure of this compound was proved to be galactosylglycerol, namely 2, 3-dihydroxypropyl (α-D-galactopyranosyl)-(1 → 6)-β-D- galactopyranoside (Fig. 2). The respective ^{1}H and ^{13}C NMR data are given in Table 2.

The ^{1}H and ^{13}C NMR signals were assigned according to DEPT, $^{1}H,^{1}H$ COSY, $^{1}H,^{1}H$ NOESY and $^{1}H,^{13}C$ correlation experiments (HETCOR, HSQC, HMBC). Configuration and glycosidic linkages (α or β) were determined by means of HMBC and NOESY spectra. For example, the following characteristic correlations, besides others, were found: in the HMBC spectrum C-1(A) with H-1'a and H-1'b; C-1(B) with H-6a(A) and H-6b(A); C-1' with H-1(A), and in the $^{1}H,^{1}H$ NOESY spectrum, besides others, cross peaks for the protons H-1(A) with H-1'a, H-1'b, H-3(A) and H-5(A); H-1(B) with H-6a(A) and H-6b(A); H-3(B) with H-5(B).

The structure was also proved by high-resolution mass spectrometry (HRMS). HRMS was in accordance to the structure of galactosylglycerol showing the $[M+Na]^{+}$ peak: HRMS of $C_{15}H_{28}O_{13}Na$ (M + Na) 439.14311, calculated 439.14221 (data not shown). By using a new developed HPLC method it was possible to

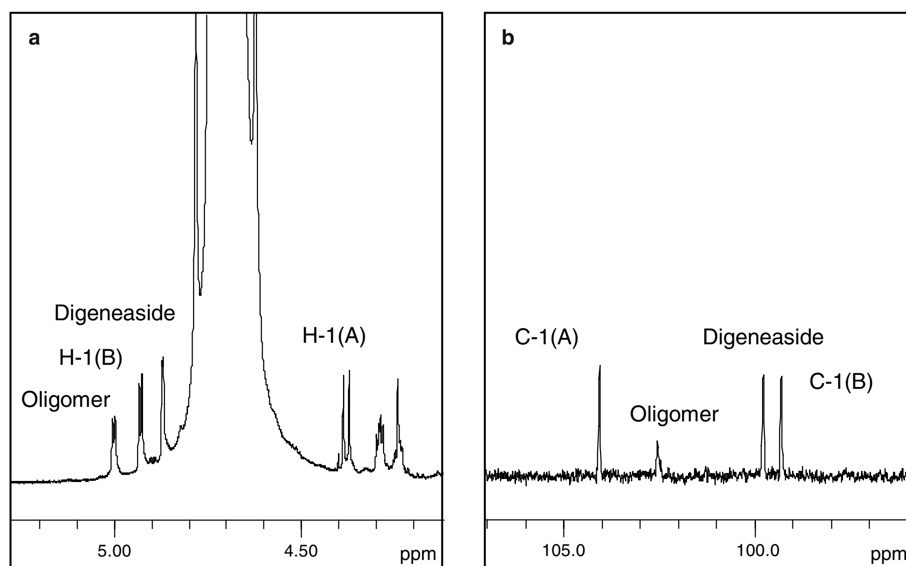

Fig. 1a,b ^{1}H (**a**) and ^{13}C NMR spectra (**b**) of the anomeric protons and carbon atoms of *H. barbatum* (2926) in D_2O solution (Ring A: $\delta_{H\text{-}1}$ = 4.38 ($J_{H\text{-}1,H\text{-}2}$ = 8.0 Hz), $\delta_{C\text{-}1}$ = 104.1; Ring B: $\delta_{H\text{-}1}$ = 4.93 ($J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz), $\delta_{C\text{-}1}$ = 99.3; Digeneaside: $\delta_{H\text{-}1}$ = 4.88 ($J_{H\text{-}1,H\text{-}2}$ = 1.6 Hz), $\delta_{C\text{-}1}$ = 99.7)

Table 2 ^{1}H and ^{13}C NMR chemical shifts δ (in ppm) and coupling constants J (in Hz) (in parentheses) of the digalactosylglycerol from *H. barbatum* (2926) in D_2O solution

		Gal A[a]	Gal B[b]
δ_{1H}	H-1	4.38 ($J_{1,2}$ = 8.0)	4.93 ($J_{1,2}$ = 3.5)
	H-2	3.50 ($J_{2,3}$ = 9.8)	3.78 ($J_{2,3}$ = 10.0)
	H-3	3.62 ($J_{3,4}$ = 3.5)	3.80 ($J_{3,4}$ = 3.0)
	H-4	3.92	3.93
	H-5	3.85	3.92
	H-6a	3.85	
	H-6b	3.67	3.70 (2H)
	H-1′a	3.83	
	H-1′b	3.71	
	H-2′	3.88	
	H-3′a	3.62	
	H-3′b	3.55 ($J_{3'a,3'b}$ = 11.6)	
δ_{13C}	C-1	104.1	99.3
	C-2	71.8	69.3
	C-3	73.6	70.4
	C-4	69.7	70.2
	C-5	74.0	72.0
	C-6	67.4	62.2
	C-1′	71.9	
	C-2′	71.4	
	C-3′	63.4	

[a]Gal A: Gal(β1–$OCH_2CH(OH)CH_2OH$)
[b]Gal B: Gal(α1–6)Gal.

separate and quantify both low molecular weight carbohydrates (Fig. 3). Under the chromatographic conditions described digalactosylglycerol and digeneaside showed retention times of 13.3 min and 13.8 min, respectively, well apart from each other to guarantee full separation of both compounds. The concentrations of digeneaside among all strains tested ranged from 131.6 mmol kg^{-1} to 539.6 mmol kg^{-1} DW, although most cultures exhibited amounts below 200 mmol kg^{-1} DW. While *H. barbatum* (2926) from the Philippines contained 221.7 mmol digalactosylglycerol kg^{-1} DW, *H. heterocystideum* (3985) from Western Australia showed 438.7 mmol kg^{-1} DW of this new compound (Table 1).

H. barbatum was treated with salinities ranging from 15 psu to 60 psu. Plants appeared viable between 20 psu and 50 psu, as reflected by the formation of some new small branches during the experiment, and plants retaining theit typical dark red coloration (data not shown). In contrast, at 15 psu and 60 psu plants bleached and died within the 3 days of incubation (data not shown). The digeneaside concentration remained almost unchanged between 25 psu and 50 psu at about 210 mmol kg^{-1} DW (Fig. 4). However, a decrease in salinity from 25 psu to 20 psu was accompanied with a decline in the digeneaside content by >50 mmol kg^{-1} DW. In contrast to digeneaside, the digalactosylglycerol concentration strongly, and almost linearly, increased with increasing salinities from 70 mmol kg^{-1} DW at 20 psu to 215 mmol kg^{-1} DW at 45 psu (Fig. 4). However, the highest salinity tested did not lead to further accumulation of digalactosylglycerol.

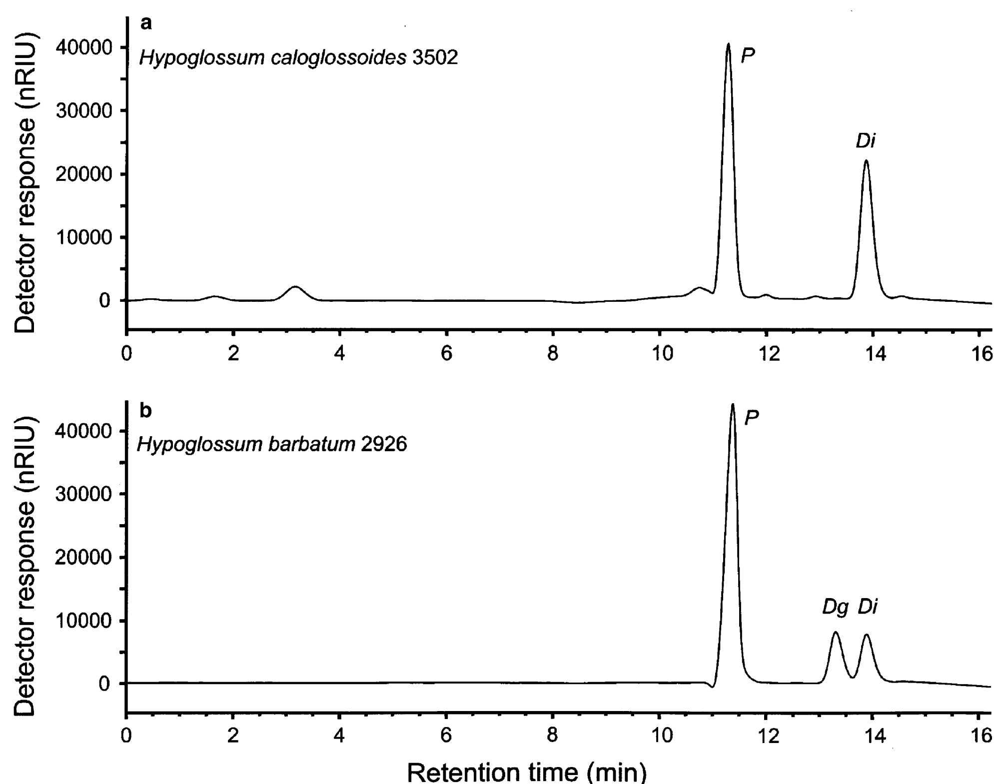

Fig. 3a,b HPLC chromatograms obtained from ethanolic extracts of (**a**) *H. caloglossoides* 3502 (**a**) and *H. barbatum* 2926 (**b**)containing different low molecular weight carbohydrate patterns. For both samples identical conditions were used and low molecular weight carbohydrates identified with a refractive index detector. P, polysaccharides (cell wall carbohydrates and florideenstarch); Dg, digalactosylglycerol at 13.3 min; Di, digeneaside at 13.8 min

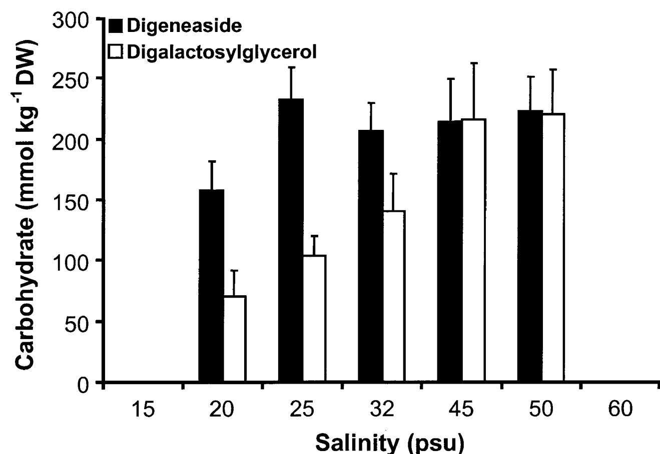

Fig. 4 The effect of salinity on the intracellular concentrations of digeneaside and digalactosylglycerol in *H. barbatum* 2926. Plants were treated for 72 h with salinities ranging from 15 to 60 psu. However, at 15 psu and 60 psu *Hypoglossum* started to bleach and finally to die over the course of the experiment. Data are expressed as mean value ± SD ($n = 4$)

Discussion

Although digeneaside is known as the major low molecular weight carbohydrate synthesized in most members of the Ceramiales (Kremer and Vogl 1975), reliable quantitative data on this compound were missing to date. Kirst (1980) quantified in the red algae digeneaside using an enzyme assay based on the hydrolytic cleavage of this heteroside using strong acid followed by the enzymatic determination of the resulting glyceric acid. However, this approach also cleaves various cellular and cell-wall macromolecules (e.g. polysaccharides) that may interfere with the assay or overestimate the contents. In recent years various ^{13}C-NMR studies have been undertaken on digeneaside patterns in red algae, that proved to be a reliable qualitative tool (Karsten et al. 1994, 1995a, 1999). However, accurate quantitative information could not be obtained from this technique, because the NMR is rather insensitive, time consuming, and needs a relatively high amount of biomass compared to chromatographic methods. Therefore, the HPLC method described here for the analysis of digeneaside provides for the first time a very simple means for quantitative measurement of this compound. Another problem related to digeneaside is the fact that this heteroside is commercially not available as standard, and hence has to be isolated and purified from a Ceramialean red alga.

The digeneaside concentrations measured in various *Hypoglossum* strains (131.6–539.6 mmol kg^{-1} DW) are much higher compared to the NMR data on *Caloglossa leprieurii* (15–30 mmol kg^{-1} DW Karsten et al. 1994) and enzyme assay data on various red algal taxa (29–79 mmol kg^{-1} DW recalculated data from Kirst 1980). Although Kirst and Bisson (1979) reported an almost two-fold accumulation of digeneaside in *Centroceras clavulatum* (C. Agardh) Montagne after increasing the salinity from 26 psu to 51 psu, these authors considered the contribution of this heteroside to the internal osmotic potential of the cells as low and therefore physiologically unimportant. In *Caloglossa leprieurii*, in contrast to the main organic osmolyte mannitol, the digeneaside concentration was unaffected after exposure to a range of salinities (Karsten et al. 1994). The data on the salt shock experiment with *H. barbatum* (2926) fully confirm these results, that is, the digeneaside values remained almost unchanged between 25 psu and 50 psu. Therefore, neither published work nor the present study indicate digeneaside to play more than a minor role in osmotic acclimation. In addition, the metabolism of this heteroside, that is, the anabolic and catabolic pathways are still unresolved, and the biological function remains unknown.

In contrast to digeneaside, digalactosylglycerol showed a >3-fold increase in concentration with rising salinities supporting its role as an organic osmolyte. Compared with other heterosides this metabolite is chemically more closely related to D-/L-isofloridoside known from members of the Bangiales where the glycerol moiety is also glycosidically linked via the C1-atom to the anomeric carbon of the respective galactose (Karsten et al. 1993; Karsten 1999). D- and L-isofloridoside play a major role in osmotic acclimation of various algal taxa (Kauss 1977; Karsten et al. 1993), which well supports this function also for digalactosylglycerol. Since floridoside and its isomeric forms also act as compatible solutes in the cytoplasm, that is, as organic compounds stabilizing enzyme systems and other biomelecules under hypersaline conditions (Kirst 1990), it is reasonable to assume this second function for digalactosylglycerol too.

Although the metabolic pathways for the biosynthesis of floridoside in red algae are well known (Kremer and Kirst 1981), those for D- and L-isofloridoside in this algal group are unstudied. The same is true for the biosynthesis of this new digalactosylglycerol in *H. barbatum* (2926) and *H. heterocystideum* (3985). A literature search for this compound indicated that digalactosylglycerol represents an important fragment of uncharged glycolipids in various types of biological membranes (Bishop et al. 1985), and hence is strongly involved in the fatty acid metabolism of plants (Vishwanath et al. 1996). However, the concentrations of glycolipids and their bound digalactosylglycerol are usually low in the cell. Only one reference was found for any plant where a free digalactosylglycerol content of only 0.1% of the dry weight in rapeseed meal was reported (Bengtsson 1985). In *Hypoglossum* 2926 and 3985 significantly higher digalactosylglycerol concentrations of 9 and 19% of the DW, respectively, were measured. From the data shown it is clear, that this compound represents a new, and physiologically important low molecular weight carbohydrate for members of this red algal genus, which calls for biochemical studies on the anabolic and catabolic pathways.

H. barbatum 2926 bleached and died after 3 days treatment with salinities of 15 psu and 60 psu, indicating a rather limited halotolerance. In contrast, related species of the genus *Caloglossa* which also belong to the Delesseriaceae, exhibited a broad growth response between 5.3 and 70 psu which is characteristic for euryhaline macroalgae (Karsten and West 1993). The smaller range of salinities which can be tolerated by *H. barbatum* can be explained by its predominant occurrence in the sublitoral zone (*H. hypoglossoides* grows at about 10 m depth; Horta et al. 2003). In deeper waters the environmental factors, particularly salinity, are much more stable compared to the eulitoral zone, and hence there is no requirement to develop physiological, mainly energy-consuming mechanisms supporting euryhalinity.

Although only seven of 22 recognized *Hypoglossum* taxa were studied (Wynne et al. 1989), it is clear that at least two low molecular weight carbohydrate patterns exist. One group including *Hypoglossum* sp. (2403), *H. tenuifolium* (3386), *H. rhizophorum* (3388), *H. caloglossoides* (3497, 3499, 3500, 3502, 3503) and *H. hypoglossoides* (4500) contains only digeneaside and the other group with *H. barbatum* (2926) and *H. heterocystideum* (3985) contains digeneaside plus digalactosylglycerol (Table 1). There seems to be even a biogeographic connection. While both species from The Philippines and Western Australia exhibited this new compound, the remaining species from Mexico, Puerto Rico, Brazil and French Polynesia contained digeneaside only. Since most of these cultures were grown for many years under identical laboratory conditions, genotypic rather than environmental and phenotypic differences may be expected. Similar differences in low molecular weight carbohydrate patterns exist in geographically distinct populations of *Bostrychia tenuissima* (Rhodomelaceae, Ceramiales) which form the polyols dulcitol and sorbitol (Karsten et al. 1995b). In Australia all populations south of 34°S contain sorbitol, whereas those north of 34°S exhibit both sorbitol and dulcitol. Molecular investigations revealed that those populations with only dulcitol have rbcL/rbcS spacer H1 haplotype, whereas those with both dulcitol and sorbitol have H2 and H3 (Zuccarello et al. 1999), indicating a genetic basis of the different polyol patterns observed. However, similar studies on *Hypoglossum* are lacking.

Acknowledgements The authors thank Oliver Nixdorf (Alfred-Wegener-Institut for Polar and Marine Research, Bremerhaven, Germany) for providing the digeneaside standard and the HPLC method, as well as Solvig Görs and Sabine Stolle for technical support. This research is partially supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) to UK (Ka 899/8-1/2). We also thank Sylvia Earle (National Geographic Society, Washington DC, USA), Hilconida Calumpong (Silliman University, Phillipines), David Ballantine (University of Puerto Rico), Jeff Shima (Victoria University of Wellington, New Zealand), Nair Nokoya (Universidade de Sao Paulo, Brazil) and John Huisman (Murdoch University, Australia) for collecting many specimens for culture work. Mike Wynne (University of Michigan, Ann Arbor, USA) provided very helpful comments on the species names for the various culture isolates. This project has been supported in part by Australian Research Council small grants SG0935526 (1994) and S19812824 (1998) as well as Australian Biological Resources Study grant (2002–2005) to JAW.

References

Barrow KD, Karsten U, King RJ (1993) Isolation of isoethionic acid from the marine red alga *Ceramium flaccidum*. Phytochemistry 34:1429–1430

Barrow KD, Karsten U, King RJ, West JA (1995) Floridoside in the genus *Laurencia* (Rhodomelaceae: Ceramiales): a chemosystematic study. Phycologia 34:279–283

Bengtsson L (1985) Improvement of rapeseed quality through breeding for high protein content. Swedish University of Agricultural Science, Svalöv, Sweden

Bishop DG, Sparace SA, Mudd JB (1985) Biosynthesis of sulfoquinovosyldiacylglycerol in higher plants—the origin of the diacylglycerol moiety. Arch Biochem Biophys 240:851–858

Freshwater DW, Fredericq S, Butler BS, Hommersand MH, Chase MW (1994) A gene phylogeny of the red algae (Rhodophyta) based on plastid rbcL. Proc Natl Acad Sci USA 91:7281–7285

Garbary DJ, Gabrielson PW (1990) Taxonomy and evolution. In: Cole KM, Sheath RG (eds) Biology of the Red Algae. Cambridge University Press, Cambridge, pp 477–498

Horta PA, Yokoya NS, Guimaraes S, Bacci DS, Oliveira EC (2003) Morphology, reproduction and development of *Hypoglossum hypoglossoides* (Stackhouse) Collins& Hervey (Ceramiales, Rhodophyta) from the south and southeastern Brazilian coast. Revista Brasil Bot 26:453–460

Karsten U (1999) Seasonal variation in heteroside concentrations of field-collected *Porphyra* species (Rhodophyta) from different biogeographic regions. New Phytol 143:561–571

Karsten U, West JA (1993) Ecophysiological studies on six species of the mangrove red algal genus *Caloglossa*. Aust J Plant Physiol 20:729–739

Karsten U, West JA, Zuccarello G (1992a) Polyol content of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae, Rhodophyta) from field and culture. Bot Mar 35:11–19

Karsten U, West JA, Mostaert A, King RJ, Barrow KD, Kirst GO (1992b) Mannitol in the red algal genus *Caloglossa* (Harvey) J Agardh. J Plant Physiol 140:292–297

Karsten U, Barrow KD, King RJ (1993) Floridoside, L-isofloridoside, and D- isofloridoside in the red alga *Porphyra columbina*. Plant Physiol 103:485–491

Karsten, U, Barrow KD, Mostaert AS, King RJ, West JA (1994) 13C- and 1H-NMR studies on digeneaside in the red alga *Caloglossa leprieurii*-a re-evaluation of its osmotic significance. Plant Physiol Biochem 32:669–676

Karsten U, Bock C, West JA (1995a) 13C-NMR spectroscopy as a tool to study organic osmolytes in the mangrove red algal genera *Bostrychia* and *Stictosiphonia* (Ceramiales). Phycol Res 43:241–247

Karsten U, Bock C, West JA (1995b) Low molecular weight carbohydrate patterns in geographically different isolates of the eulittoral red alga *Bostrychia tenuissima* from Australia. Bot Acta 108:321–326

Karsten U, Barrow KD, Nixdorf O, King RJ (1996) The compability with enzyme activity of unusual organic osmolytes from mangrove red algae. Aust J Plant Physiol 23:577–582

Karsten U, West JA, Zuccarello GC, Nixdorf O, Barrow KD, King RJ (1999) Low molecular weight carbohydrate patterns in the Bangiophyceae (Rhodophyta). J Phycol 35:967–976

Karsten U, West JA, Zuccarello GC, Engbrodt R, Yokoyama A, Hara Y, Brodie J (2003) Low molecular weight carbohydrates of the Bangiophycidae (Rhodophyta). J Phycol 39:584–589

Kauss H (1977) Biochemistry of osmotic regulation. Int Rev Biochem 13: 119–140

Kirst GO (1980) Low mw carbohydrates and ions in Rhodophyceae: quantitative measurement of floridoside and digeneaside. Phytochemistry 19:1107–1110

Kirst GO (1990) Salinity tolerance of eukaryotic algae. Annu Rev Plant Physiol Plant Mol Biol 41:21–53

Kirst GO, Bisson MA (1979) Regulation of turgor pressure in marine algae:ions and low-molecular-weight organic compounds. Aust J Plant Physiol 6:539–556

Kremer BP (1978) Patterns of photoassimilatory products in Pacific Rhodophyceae. Can J Bot 56:1655–1659

Kremer BP, Kirst GO (1981) Biosynthesis of 2-O-D-glycerol-D-galactopyranoside (floridoside) in marine Rhodophyceae. Plant Sci Lett 23:349–357

Kremer BP, Vogl R (1975) Zur chemotaxonomischen Bedeutung des [14C] Markierungs-musters bei Rhodophyceen. Phytochem 14:1309–1314

Meng J, Rosell KG, Srivastava LM (1987) Chemical characterization of floridosides from *Porphyra perforata* . Carbohydr Res 161:171–180

Pueschel CM (1987) Absence of cap membranes as a characteristic of pit plugs of some red algal orders. J Phycol 23:150–156

Vishwanath BS, Eichenberger W, Frey FJ, Frey BM (1996) Interaction of plant lipids with 14 kDa phospholipase A82 enzymes. Biochem J 320:93–99

West JA, McBride DL (1999) Long-term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). Hydrobiol 398(399):101–111

Wynne MJ, Price IR, Ballantine DL (1989) Distinctions between *Hypoglossum barbatum* Okamura, *H minimum* Yamada and *H simulans* sp nov (Delesseriaceae, Rhodophyta). Phycologia 28:28–38

Zuccarello GC, West JA, Karsten U, King RJ (1999) Molecular relationships within *Bostrychia tenuissima* (Rhodomelaceae, Rhodophyta). Phycol Res 47:81–85