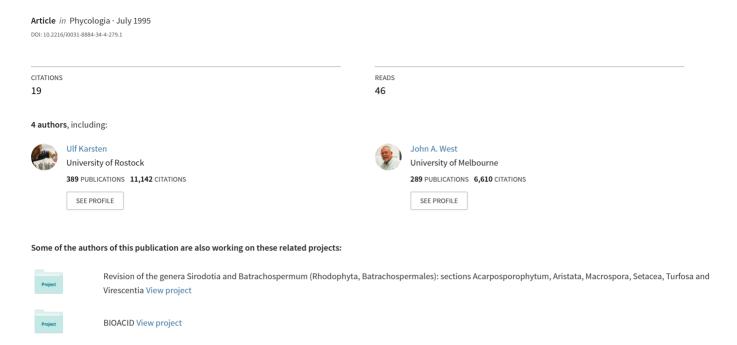
Floridoside in the genus Laurencia (Rhodomelaceae: Ceramiales) - A chemosystematic study



Floridoside in the genus *Laurencia* (Rhodomelaceae: Ceramiales) – a chemosystematic study

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The heteroside floridoside has been recorded in 21 specimens of Laurencia, as well as in some closely related species: Osmundea hybrida (A.P. de Candolle) Nam, O. pinnatifida (Hudson) Stackhouse, O. spectabilis (Postels et Ruprecht) Nam, O. truncata (Kützing) Nam et Maggs and Chondria capensis (Harvey) Askenasy (Rhodomelaceae, Ceramiales). The occurrence of this carbohydrate in the Ceramiales negates the proposition that the presence of floridoside is a chemotaxonomic marker only for orders of the Rhodophyta other than the Ceramiales. Digeneaside, which is known as a characteristic photo-assimilatory product only in representatives of the Ceramiales, was not detected in any of the species investigated, including five species in which it was previously detected; its previously proposed high diagnostic value is negated and its reported occurrence in these species is questioned.

INTRODUCTION

Members of all orders of Rhodophyta other than the Ceramiales synthesize the heteroside floridoside (a-D-galactopyranosyl-(1-2)-glycerol) and/or isofloridoside as a major product of photosynthesis and a storage compound. In the Ceramiales digeneaside (a-D-mannopyranosy1-(1-2)-glycerate) is accumulated and has been regarded as a chemotaxonomic character (Kirst 1980; see also Kremer & Vogl 1975; Kremer 1978a). Court (1980) investigated the photosynthetic properties of two algae in the order Ceramiales, Laurencia spectabilis Postels et Ruprecht and the alleloparasite Janczewkia gardneri Setchell et Guernsey, and found that the 14C label was incorporated into a range of compounds including both floridoside and isofloridoside; no evidence was found for digeneaside, either labelled or unlabelled. Court's results directly contradicted the general conclusions of Kremer & Vogl (1975) and Kremer (1978a, b). He provided limited data indicating that the discrepancy is unlikely to be due to seasonal or local environment differences, and suggested that a more discriminating and reliable study needed to be undertaken on the assimilation products of Laurencia and related genera. Very recently some Laurencia species including L. spectabilis have been transferred to the resurrected genus Osmundea (Nam et al. 1994). These authors recognized Osmundea as different from Laurencia, due to anatomical characters of vegetative and male reproductive structures and tetrasporangial development.

In a routine ¹³C-nuclear magnetic resonance spectroscopy (¹³C-NMR) investigation of potential osmoregulatory compounds in a range of red algae, we noted the consistent presence of floridoside in different isolates of *Laurencia fili*-

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formis (C. Agardh) Montagne and therefore undertook a survey of members of this genus and several closely related species, which clarifies the issue raised by Court (1980).

MATERIALS AND METHODS

Plant material

All details of the collection locations and dates of the algae used for chemical analysis are presented in Table 1. Most species were collected in the field at low tide and subsequently air-dried in the sun prior to chemical analysis. Other samples were collected by colleagues around the world and mailed as dried material. In addition, some herbarium specimens were included in the study.

¹³C-NMR methodology

For the ¹³C-NMR measurements usually 100–500 mg of algal dry weight (DW) was extracted in 5 ml 70% ethanol (v/v) for 3 h in a waterbath at 70°C. After centrifugation at 5000 g, the supernatant was evaporated to dryness and redissolved in 0.5 ml D₂O (99.98%) for NMR spectroscopy. For some samples the extraction was repeated three times to check the efficiency of the procedure. Occasionally there was so much water-insoluble material present in the evaporated extracts that it was necessary to carry out a preliminary trituration with ether to obtain a sample suitable for NMR. In one case (Laurencia filiformis) a large-scale extraction was carried out with 1 kg of algal fresh weight. The procedure was essentially the same except that, after evaporation, the residue of the alcoholic extract was partitioned between dichloromethane and water, and the aqueous phase then evaporated. ¹³C-NMR

Table 1. The floridoside concentration in field and herbarium species of Laurencia and Osmundea from different geographic regions and in Chondria capensis from South Africa. Data are expressed as mean \pm standard deviation (n=4) in mmol kg⁻¹ DW. Some samples could only be used for one chemical analysis, due to small amounts of material

Species	Location	Date	Collector	Floridoside
Laurencia arbuscula Sonder	Williamstown, Victoria, Australia	5 Aug. 93	J.A. West	163.0 ± 32.0
L. arbuscula	Williamstown, Victoria, Australia	5 Aug. 93	J.A. West	120.6 ± 19.1
L. botryoides (Turner) Gaillon	Eaglehawk, Tasmania	Nov. 1924	Herbarium Botanical Garden Sydney	26.1
L. brongniartii J. Agardh	Coloundra, Australia	Aug. 1922	Herbarium Botanical Garden Sydney	44.1
L. filiformis (C. Agardh)	Maroubra, Sydney, Australia,	5 Nov. 92	U. Karsten	289.0 ± 24.5
Montagne L. filiformis	Maroubra, Sydney, Australia	24 Nov. 92	U. Karsten	335.5 ± 55.8
L. filiformis L. filiformis	La Perouse, Sydney, Australia	13 Oct. 92	R. DeNys	159.5 ± 4.1
L. filiformis	Williamstown, Victoria, Australia,	5 Aug. 93	J.A. West	109.6 ± 9.0
L. filiformis forma heteroclada (Harvey) Saito et Womersley	Twelve Apostles, Victoria, Australia,	19 Jan. 93	U. Karsten	1303.5 ± 232.3
L. filiformis forma heteroclada	Robe, South Australia	20 Jan. 93	U. Karsten	114.7
L. flagellifera J. Agardh	Brava Beach, Sao Paulo, Brazil	15 Sep. 93	M. Fujii	20.7 ± 13.1
L. furcata sp. nov. Fujii	Capula, Espiritu Santo, Brazil	30 Jun. 92	M. Fujii	25.3
L. inplicata J. Agardh	Geoffrey Bay, Magnetic Island, Australia	26 Sep. 92	I. Price	417.5 ± 69.7
L. majuscula (Harvey) Lucas	Williamstown, Victoria, Australia	5 Aug. 93	J.A. West	146.0 ± 3.1
L. obtusa (Hudson) Lamouroux	Dumaguete City, the Philippines	10 Jun. 93	N. Calumpong	5.3 ± 2.9
L. obtusa	Doorin Point, Donegal, Ireland	15 Oct. 89	C. Maggs	46.0 ± 24.0
L. obtusa	Hainan, Peoples Republic of China	Feb. 92	B. Bischoff	78.7
L. osmunda (S.G. Gmelin) Maggs et Hommersand	Torbay, Devon, England	10 Mar. 89	C. Maggs	60.3 ± 51.3
L. pacifica Kylin	Stillwater Cove, California, USA	5 May 74	J.A. West	40.3 ± 10.2
L. pacifica	Stillwater Cove, California, USA	9 May 93	J.A. West	206.1 ± 20.7
L. papillosa (C. Agardh) Greville	Dumaguete City, the Philippines	10 Jun. 93	N. Calumpong	< 0.5
L. patentiramea (Montagne) Kützing	Dumaguete City, the Philippines	10 Jun. 93	N. Calumpong	< 0.5
L. poiteaui (Lamouroux) Howe L. pyramidalis Bory ex Kützing	La Parguera, Puerto Rico West Point, Isles of Scilly, England	2 Jun. 93 26 Sep. 88	D. Ballantine C. Maggs	$125.8 \pm 9.2 \\ 35.4 \pm 26.0$
L. scoparia J. Agardh	Brava Beach, Sao Paulo, Brazil	15 Sep. 93	M. Fujii	30.5 ± 28.5
L. similis Nam et Saito	Geoffrey Bay, Magnetic Island, Australia	26 Sep. 92	I. Price	228.7 ± 34.9
L. subopposita (J. Agardh) Setchell	Stillwater Cove, California, USA,	9 May 93	J.A. West	386.6 ± 34.2
L. tenera Tseng	Kissing Point, Townsville, Australia	27 Aug. 92	I. Price	386.2 ± 56.1
L. tumida Saito et Womersley	Williamstown, Victoria, Australia	5 Aug. 93	J.A. West	60.6 ± 17.4
L. sp.	Robe, South Australia	20 Jan. 93	U. Karsten	120.0
L. sp.	Ishigaki, Japan	10 Sep. 93	E. Coppejans (Herbarium EC 9989)	207.1 ± 42.4
Osmundea hybrida (A.P. de Candolle) Nam	Ballycastle, Northern Ireland	7 Feb. 89	C. Maggs	154.5 ± 48.0
O. pinnatifida (Hudson) Stackhouse	Island Magee, Antrim, Northern Ireland	20 Feb. 89	C. Maggs	140.2 ± 40.4
O. spectabilis (Postels et Ruprecht) Nam	Duxbury Reed, California, USA	10 Dec. 77	Herbarium University of California	252.8 ± 51.3
O. spectabilis	Goverment Point, California, USA	27 Dec. 47	Herbarium University of California	35.1 ± 33.2
O. spectabilis	Pidgeon Point, California, USA	9 Apr. 93	J.A. West	226.7 ± 35.2
O. spectabilis	Agate Beach, California, USA	7 May 93	J.A. West J.A. West	138.4 ± 18.6
O. spectabilis	Stillwater Cove, California, USA	9 May 93	J.A. West	523.3 ± 60.1
O. truncata (Kützing) Nam et Maggs	Lyme Regis, Dorset, England	26 Apr. 90	C. Maggs	302.2 ± 19.7
Chondria capensis (Harvey) Askenasy	Cape Province, South Africa	6 Jul. 93	R.J. King	56.2 ± 43.7

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 $3.5~\mu s$ 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 contained in

spinning 5 mm tubes and spectra were run at 27°C and referenced from added dioxane (67.4 ppm).

Floridoside quantification

Floridoside was quantified by gas-liquid chromatography (GLC) and high-pressure liquid chromatography (HPLC). For both measurements the algal samples (10–15 mg DW) were extracted in 1 ml 70% ethanol (v/v) for about 3 h in a waterbath at 70°C. These were then centrifuged for 5 min at 5000 g.

For the GLC analysis, 10 µl of the internal standard myoinositol (1 mg ml⁻¹) was added to $30-100 \mu l$ of the sample supernatant or to a standard of known concentration, and evaporated to dryness under a stream of dry air. The residue was re-dissolved in 150 μ l pyridine and 20 μ l of the silylation reagent N-trimethylsilylimidazole (TMSI) (Pierce Chemical Co., Rockford, USA), shaken for 30 s and left overnight. The silvlated samples were analysed directly by GLC. The determinations were performed with a Hewlett-Packard 5890A GLC equipped with a flame-ionization detector and connected to an automatic injector 7633. Data were analysed and processed using a Hewlett-Packard GC ChemStation. A fused-silica capillary column (BP5, 25 m × 0.32 mm ID) was used, with hydrogen as the carrier gas at a head pressure of 10 psi. Floridoside was separated from other carbohydrates with a temperature program: the oven temperature was kept for 1 min at 150°C following injection (1 μ l) 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 HPLC measurements, $700 \,\mu l$ of the supernatant was evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H, Bachofer Ltd, BRD). Dried samples were redissolved in $700 \,\mu l$ distilled water, purified through a 0.45- μ m syringe microfilter and directly utilized for analysis. Floridoside was quantified by HPLC with a refractive index detector. All analytical conditions are described in detail by Karsten *et al.* (1991).

Calibration of both chromatography systems 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 as reported by Karsten *et al.* (1993a). The floridoside contents are expressed as concentration on a dry-weight basis.

RESULTS

Except for the herbarium specimens of Laurencia, all other plants listed in Table 1 were investigated by ¹³C-NMR spectroscopy. The spectra of all samples showed nine major resonances at 61.3, 61.9, 62.3, 69.3, 70.1, 70.2, 71.9, 79.6 and 98.9 ppm, which are characteristic for floridoside (Karsten et al. 1993b). Digeneaside, which gives typical resonances at 61.8, 63.9, 67.6, 70.9, 71.2, 73.8, 78.8, 99.3 and 177.7 ppm (Karsten et al. 1994) could not be detected in any of the samples tested. The quantitative data for floridoside in all species of Laurencia and Osmundea, as well as in Chondria capensis, are provided in Table 1. Floridoside was detected in all algae examined, but there was considerable variation even within single species. The concentrations ranged from trace amounts of <0.5 mmol kg⁻¹ DW (e.g. L. papillosa

(C. Agardh) Greville) up to 1303.5 mmol kg⁻¹ DW in L. filiformis forma heteroclada (Harvey) Saito et Womersley (Table 1). Usually we recorded 800-1000 scans on each sample and typically the signal to noise ratio for the floridoside resonances was 5-10 to 1. Under these conditions it would not be possible to detect digeneaside at amounts of 0.5% or less of floridoside. Therefore selected samples were run for much longer times and signal to noise ratios of 50-100 to 1 were obtained. Using a larger line broadening factor also increased the signal to noise ratio and hence the sensitivity of detection. This was performed for samples of L. filiformis, L. majuscula (Harvey) Lucas and L. tumida Saito et Womersley, and the results indicate that if digeneaside is present in these species it must be at concentrations of less than 0.5% of that of floridoside. To check further, a largescale extraction of L. filiformis was carried out and part of this extract run at a much higher concentration. Again no digeneaside could be detected and, if any is present, it must be at concentrations less than 0.05-0.1% of floridoside. Many of the extracts showed other resonances with peaks in the range between 25 and 55 ppm and these were very speciesspecific. Preliminary ion exchange chromatography has shown that these compounds are retained on sulphonic acid ion exchange resins and so must have certain basic structural groups. In some cases these resonances were considerably larger than those for floridoside and these compounds may contribute significantly to osmotic adjustment. We also checked very carefully for the presence of any of the isofloridoside isomers. None were detected in any samples examined in this work.

DISCUSSION

The use of the presence or absence of various chemical constituents in algal taxonomy has a long history: by the middle of the nineteenth century W.H. Harvey was already using a classification of the major groups of algae based largely on colour. Subsequent studies have established the complement of pigments diagnostic of the different algal divisions as well as the major reserve substances and the organic cell-wall components (Percival 1979; Kremer 1980; Rowan 1989).

Within the red algae, the diversity of photosynthetic products, some of which are known to function as compatible solutes in osmoregulation (Kirst 1990), have been considered to be of taxonomic use for this group (Kremer 1978a, 1980). In all orders other than the Ceramiales the main compounds reported are the heterosides, floridoside and/or isofloridoside. In the Ceramiales, digeneaside is usually present and has been thought to be of high chemosystematic value (Kirst 1980; Kremer & Kirst 1982). Kremer & Vogl (1975) and Kremer (1978) detected digeneaside in Osmundea hybrida (as Laurencia hybrida), O. pinnatifida (as Laurencia pinnatifida), Laurencia obtusa (Hudson) Lamouroux and L. subopposita (J. Agardh) Setchell after ¹⁴C-labelling followed by thin-layer chromatography and autoradiography. Kirst (1980) measured digeneaside in L. obtusa using an enzymatic test which is based on an acid hydrolysis and the subsequent oxidation of free glyceric acid to hydroxy pyruvic acid by NAD+ and glyceric acid dehydrogenase. We included these Laurencia and Osmun-

dea species in our study and were unable to find detectable traces of digeneaside by using the powerful and accurate 13C-NMR technique. Instead, floridoside was present in all samples tested. It seems possible that the earlier data were due to methodological inaccuracies. The occurrence of floridoside also in Osmundea and Chondria capensis, i.e. in further genera of the Ceramiales, points to the possibility that more representatives of the order may contain this heteroside, therefore the proposed high diagnostic value of digeneaside for the Ceramiales has to be revised. Besides the occurrence of floridoside in Laurencia, other biochemical exceptions are known within the Ceramiales. The members of the Bostrychioideae (Rhodomelaceae, Ceramiales), for example, are characterized by the sugar alcohols dulcitol and sorbitol, which are otherwise very unusual in red algae (Karsten et al. 21992a). Both polyols are strongly involved in osmotic acclimation (Karsten et al. 1993b). In most Bostrychia species both compounds occur; but in the related genus Stictosiphonia, dulcitol is lacking (Karsten et al. 1992a). Recently, we have shown that in Caloglossa (Delesseriaceae, Ceramiales) the main low molecular weight carbohydrate is mannitol, which also appears to function in osmoregulation (Karsten et al. 1992b). Among the macroalgae, the presence of mannitol had been regarded as an important chemotaxonomic marker for the brown algae, despite earlier reports of mannitol in the red alga Neorhodomela larix (Turner) Masuda (as Rhodomela larix (Turner) C. Agardh) (Whyte & Southcott 1970). In Ceramium flaccidum (Kützing) Ardissone (Ceramiaceae, Ceramiales), isethionic acid (2-hydroxyethane sulphonic acid) has been isolated and purified (Barrow et al. 1993). In that species it occurred in higher concentrations than does digeneaside; but, to our knowledge, no obvious biological function in algae has been ascribed to it. The detection of mannitol in Caloglossa and of isethionic acid in Ceramium emphasizes the importance of the use of sophisticated analytical methods (13C-NMR along with GLC and/or HPLC). Such techniques allow for rapid and accurate screening for a range of low molecular-weight carbohydrates, betaines and similar compounds (Blunden et al. 1992). The results from these studies cited and the present one also stress the need for broad and representative sampling with taxa before generalizations can be made. Since these compounds can have a physiological role other than simply carbon and/or nitrogen storage, it may be that ecological conditions rather than phylogenetic relations are the factor(s) determining their presence. The genera Caloglossa, Bostrychia and Stictosiphonia with sugar alcohols are all characteristic for the upper littoral zone in saltmarsh and mangrove habitats and the occurrence of high concentrations of unusual compounds may indicate biochemical adaptations to that specific environment. There may also be seasonal changes in the quantitative aspects of storage products. For example, in Bangia atropurpurea (Roth) C. Agardh and Porphyra columbina Montagne, floridoside and both D- and L-isofloridoside have been recorded; but, there were significant changes in the relative and absolute amounts throughout the season and at times particular heterosides could not necessarily be detected (Karsten et al. 1993b). In both species the lowest heteroside concentrations were determined in winter, whereas in summer the values peaked. A similar seasonal effect was observed in our study for floridoside in Laurencia filiformis. In the Australian winter

(August) plants of this species exhibited a floridoside content of 109.6 mmol kg⁻¹ DW. Between October and November, the concentrations increased from 159.5 to 335.5 mmol kg⁻¹ DW (Table 1). Besides these results, however, the present study demonstrates considerable variation within *Laurencia* species and there are insufficient data to draw any general conclusions.

The data presented fit in with the observations of Court (1980) that floridoside is a major compound in the genus *Laurencia* and are directly in opposition to the generalization that its absence be considered typical of the order Ceramiales.

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