NPC Natural Product Communications

2010
Vol. 5
No. 4
581 - 585

Betaine Yields from Marine Algal Species Utilized in the Preparation of Seaweed Extracts Used in Agriculture

Gerald Blunden[a*], Peter F. Morse[b], Imre Mathe[c,d], Judit Hohmann[c], Alan T. Critchley[e] and Stephen Morrell[f]

[a]*School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth, Hampshire PO1 2DT, UK*

[b]*The Open University in the South, Oxford OX1 5HR, UK*

[c]*Department of Pharmacognosy, University of Szeged, Eötvös u. 6, 6720 Szeged, Hungary*

[d]*Institute of Botany and Ecology of the Hungarian Academy of Sciences, Alkotmány u 2, 2163 Vácrátót, Hungary*

[e]*Acadian Seaplants Limited, 30 Brown Avenue, Dartmouth, NS, B3B 1X8, Canada*

[f]*Field Studies Council, Dale Fort Field Centre, Haverfordwest, Pembrokeshire SA62 3RD, UK*

gs_blunden@dsl.pipex.com

Received: November 11th, 2009; Accepted: January 5th, 2010

Ascophyllum nodosum, and to a lesser extent, *Laminaria digitata*, *L. hyperborea* and *Fucus serratus*, are marine algal species utilized in the commercial production of seaweed extracts used in agriculture. Betaines have been shown to be important constituents of these extracts, but there appears to have been no study made on whether there are variations in the betaine contents of these species based on either the place or date of collection. Samples of each of the four species were collected from widely separated areas at different times of the year. Also, in the case of *A. nodosum*, approximately monthly collections were made from one location. The betaines detected in the various collections of the same species showed little variation, although in the case of *A. nodosum*, glycinebetaine was found as a minor constituent in some samples, but was not detected in others. Trigonelline was found in all the tested samples of the two *Laminaria* species; this is, to our knowledge, the first record of this betaine in marine algae. With the exception of trigonelline in the *Laminaria* species, the betaine yields from the various samples of *L. digitata*, *L. hyperborea* and *F. serratus* showed little variation, regardless of either the place or date of collection. The trigonelline contents of the *Laminaria* species collected at one location (Finavarra, Ireland), in particular of *L. hyperborea*, was substantially greater than those from the other places of collection. In the case of *A. nodosum*, the betaine yields from samples collected at one site (Dale, Pembrokeshire, UK) were significantly higher than those from the other places of collection, which were very similar to each other. There was no clear indication of seasonal variation in betaine yields from *A. nodosum*.

Keywords: *Ascophyllum nodosum*, *Laminaria digitata*, *Laminaria hyperborea*, *Fucus serratus*, seaweed extracts, betaines, yield variations, trigonelline.

Extracts and suspensions derived from marine brown algae have been used in agriculture and horticulture for many years, and a wide range of beneficial effects has been reported from their use [1a-b]. In Europe and North America, the species most commonly used is *Ascophyllum nodosum* (L.) Le Jolis, although other species are also utilized by some extract producers, for example, *Laminaria digitata* (Huds.) Lamour., *L. hyperborea* (Gunn.) Fosl., and *Fucus serratus* L.

Of the claims made for the use of seaweed extracts, of particular interest are those that relate to the increased resistance of the treated plants to stress conditions. Blunden *et al.* [2] suggested the possibility that betaines present in the extracts could be responsible for some of the reported effects. From *A. nodosum*, γ-aminobutyric acid betaine, ό-aminovaleric acid betaine and laminine have been reported, and from *F. serratus*, *L. digitata* and *L. hyperborea*, glycinebetaine, γ-aminobutyric acid betaine and laminine [3a-b].

Trials were conducted on the effects of treating dwarf French bean, tomato, wheat, barley, and maize plants with either a seaweed extract produced from *A. nodosum* or betaine solutions containing amounts equivalent to those present in the extract. Treatment with all the test solutions led to similar results, with significantly higher levels of chlorophyll in the treated plants in comparison with the controls [4a].

Soil application to the roots of tomato plants of a commercial *A. nodosum* extract resulted in significant reductions in the number of second-stage juveniles of the root knot nematodes, *Meloidogyne javanica* and *M. incognita* invading the roots. The three major betaines present in the extract, when applied in quantities equivalent to those in the extract, also led to significant reductions in nematode invasion. These results led to the conclusion that the betaines present in the seaweed extract play a major role in the effects observed in the treated plants [4b].

Jayaraj *et al.* found that treatment of carrot plants with an *A. nodosum* extract resulted in them showing significantly reduced disease severity caused by pathogenic fungi. The activity of certain defense-related enzymes was significantly increased in the treated plants in comparison with the controls. Treatment of the plants with the seaweed extract enhanced their disease resistance by induction of defense genes and proteins [5a]. The constituents of the seaweed extract responsible for this activity were not determined, but betaines are a possibility. Kraska and Schönbeck reported that application of very low amounts of betaines resulted in significant enhancement in the ability of treated plants to resist fungal attack [5b]. Tyihák *et al.* have also shown that application to plants of very low amounts of betaines and other methylated compounds induces an immune response to fungal attack [5c,d]. Other components of seaweed extracts have also been shown to give treated plants added resistance to stress conditions. Application of either an *A. nodosum* extract or its lipophilic fraction led to treated plants having enhanced freezing tolerance [6].

Although betaines have been shown to be important constituents of seaweed extracts, there appears to have been no study made on whether there are variations in the betaine contents of *A. nodosum*, *F. serratus*, *L. digitata* and *L. hyperborea* based on either when or where they are harvested. As a result, samples of each of the four species have been collected from a wide variety of places at different times of the year. Also, in the case of *A. nodosum*, approximately monthly collections were made from one location. The results obtained are presented in this communication.

Table 1a: Betaine yields (%, dry wt) from different collections of *Ascophyllum nodosum* and *Fucus serratus*.

Ascophyllum nodosum

Place of collection	Date of collection	gb	γ-abab	δ-avab
Helgoland, Germany	Aug. 2006	0.01	0.02	0.02
Bergen, Norway	Sept. 2006	nd	0.06	0.02
Hella, Faroe Islands	Sept. 2006	+	0.03	0.01
Emsworth, Hampshire, UK	June 2008	+	0.05	0.02
Wells-next-the-Sea, Norfolk, UK	July 2008	nd	0.02	0.02
Kimmeridge, Dorset, UK	July 2008	nd	0.07	0.05
Cobscook, Maine, USA	Aug. 2008	+	0.04	0.02
Freshwater Bay, Isle of Wight, UK	Sept. 2008	+	0.06	0.02
Letete, New Brunswick, Canada	Sept. 2008	0.02	0.03	0.02
Lobster Bay, Nova Scotia, Canada	Oct. 2008	nd	0.06	0.04
Finavarra, Co. Clare, Ireland	Dec. 2008	+	0.04	0.02
Nelon, near Brest, France	Jan. 2009	+	0.03	0.02
St Malo, Brittany, France	March 2009	+	0.07	0.02
Peel, Isle of Man	April 2009	nd	0.08	0.03

Fucus serratus

Place of collection	Date of collection	gb	γ-abab
Wembury, Devon, UK	June 2008	0.04	0.02
Kimmeridge, Dorset, UK	July 2008	0.03	0.02
Freshwater Bay, Isle of Wight, UK	Sept. 2008	0.04	0.04
Felpham, West Sussex, UK	Sept. 2008	0.03	0.03
Portsmouth Harbour, Hampshire, UK	Sept. 2008	0.02	0.03
Lobster Bay, Nova Scotia, Canada	Oct. 2008	0.04	0.03
Birling Gap, East Sussex, UK	Oct. 2008	0.05	0.01
Finavarra, Co. Clare, Ireland	Dec. 2008	0.06	0.01
Dinard, Brittany, France	March 2009	0.05	0.03
Castletown, Isle of Man	April 2009	0.03	0.02

gb = glycinebetaine; γ-abab = γ-aminobutyric acid betaine; δ-avab = δ-aminovaleric acid betaine; + = less than 0.01%; nd = not detected

The dried seaweed samples were extracted with aqueous methanol, the extracts semi-purified by passage through a column of cation exchange resin, and then examined by TLC and ^{1}H NMR spectroscopy. The betaines identified were estimated quantitatively from the semi-purified extracts using a ^{1}H NMR spectroscopic assay procedure [9].

The extracts of *A. nodosum* consistently contained γ-aminobutyric acid betaine, δ-aminovaleric acid betaine and laminine, which is in agreement with previously published work [3a]. However, in addition, glycinebetaine was detected in some of the samples, both by TLC and ^{1}H NMR spectroscopy. The content was very low and in many samples the compound could not be detected by TLC. In these samples, a small signal in the ^{1}H NMR spectrum could be observed corresponding to the N-CH_3 resonance of glycinebetaine. However, this could not definitely be assigned to the presence of glycinebetaine and so, in these cases, 'not detected' has been ascribed (Tables 1a, 2). In a recent study, MacKinnon *et al*. reported glycinebetaine as a consistent component of *A. nodosum* [10]. Glycinebetaine has been reported previously as a constituent of commercial seaweed extracts prepared

Table 1b: Betaine yields (%, dry wt) from different collections of *Laminaria hyperborea* and *L. digitata.*

Laminaria digitata

Place of collection	Date of collection	gb	γ-abab	trig
Kimmeridge, Dorset, UK	July 2008	0.05	0.03	0.001
Clarke's Cove, near Peggy's Cove, Nova Scotia, Canada	Aug. 2008	0.02	0.02	0.006
Freshwater Bay, Isle of Wight, UK	Sept. 2008	0.02	0.02	0.001
Douglas Bay, Isle of Man	Sept. 2008	0.02	+	0.004
Birling Gap, East Sussex	Oct. 2008	0.03	0.02	0.003
South Bay, Scarborough, North Yorkshire, UK	Nov. 2008	0.03	0.01	0.002
Seacombe, Devon	Dec. 2008	0.02	+	0.002
Spiddal, Co. Galway, Ireland	Jan. 2009	0.03	0.01	0.002
St Malo, Brittany, France	March 2009	0.02	0.01	0.001
Finavarra, Co. Clare, Ireland	May 2009	0.01	+	0.001

Laminaria hyperborea

Place of collection	Date of collection	gb	γ-abab	trig
Wembury, Devon, UK	June 2008	0.06	0.03	0.008
Kimmeridge, Dorset, UK	July 2008	0.02	0.01	0.006
Freshwater Bay, Isle of Wight, UK	Sept. 2008	0.02	+	0.008
Felpham, West Sussex, UK	Sept. 2008	0.02	+	0.006
Laxey Bay, Isle of Man	Sept. 2008	0.02	0.01	0.006
South Bay, Scarborough, North Yorkshire, UK	Nov. 2008	0.04	+	0.007
Nelon, near Brest, France	Jan. 2009	0.04	0.03	0.009
Cliff Cottages Beach, Dale, Pembrokeshire, UK	Jan. 2009	0.02	+	0.002
St Malo, Brittany, France	March 2009	0.02	+	0.002
Cobo Bay and Lihou Island, Guernsey, Channel Islands	March 2009	0.03	0.01	0.002

gb = glycinebetaine; γ-abab = γ-aminobutyric acid betaine; δ-avab = δ-aminovaleric acid betaine; trig = trigonelline; + = less than 0.01%.

from this species, but this was thought to be the result of contamination of the starting material with other algae, such as *Fucus* species [1]. In this current study, laminine was always present in low amount; this is consistent with the results of McKinnon *et al.* [10].

All the extracts tested of *F. serratus* contained γ-amino-butyric acid betaine, glycinebetaine and laminine, which is consistent with earlier studies of this species [3a]. As in the case of *A. nodosum*, laminine contents were low and quantitative results were not obtained.

As reported previously [11], both *L. digitata* and *L. hyperborea* contained glycinebetaine, γ-aminobutyric acid betaine and laminine, the last in small amounts. Trigonelline was also detected in all the *Laminaria* samples examined, regardless of the date and place of collection (Table 1b). In some of the ten samples from Finavarra (not shown in Table 1), the yield of trigonelline was greater than that of γ-aminobutyric acid betaine. To the best of our knowledge, this is the first record of trigonelline for a marine alga, although this compound is widely distributed in higher plants [11a, b]. Lysinebetaine was not detected in the two *Laminaria* species, but in the previous study this was only found as a very minor constituent [12]. As with the other tested species, the laminine contents were low and not quantified.

With *F. serratus* there was no significant difference in the betaine yields as a result of either collection place or date (Table 1a). The variations found in the ten samples taken at the same time of year from the same area (glycinebetaine 0.03-0.08%, mean 0.05%; γ-aminobutyric acid betaine 0.02-0.04%, mean 0.03%) were very similar to those of the collections from different places at different times of the year (glycinebetaine 0.02-0.06%, mean 0.04%; γ-amino-butyric acid betaine 0.01-0.04%, mean 0.02%).

In the case of the two *Laminaria* species studied, the collection place and date also seemed to be of little importance with respect to betaine yields With *L. hyperborea*, from the different collections made at the same time of year from the same area, glycinebetaine contents varied from 0.01 to 0.07%, (mean 0.04%), and γ-aminobutyric acid betaine from less than 0.01% to 0.03% (mean 0.01%). The equivalent values for the collections from different places at different times of the year were glycinebetaine 0.02% to 0.06% (mean 0.03%) and γ-aminobutyric acid betaine less than 0.01% to 0.03% (mean 0.01%). For *L. digitata*, from the different collections made at the same time of year from the same area glycinebetaine contents varied from 0.01 to 0.02%, (mean 0.015%), and γ-aminobutyric acid betaine from less than 0.01% to 0.03% (mean 0.02%). The equivalent values for the collections from different places at different times of the year were glycinebetaine 0.01% to 0.05% (mean 0.03%) and γ-aminobutyric acid betaine less than 0.01% to 0.03% (mean 0.015%).

However, unlike the other betaines, the content of trigonelline appeared to be considerably greater in the *Laminaria* species collected at Finavarra in May than in the samples collected elsewhere at various times of the year. This was particularly the case with *L. hyperborea*, in which the trigonelline content varied from less than 0.01% to 0.03% (mean 0.02%) in the Finavarra samples, whereas in the others, the content varied from 0.002% to 0.009% (mean 0.0055%).

For the ten *A. nodosum* samples collected in May from the Finavarra area, γ-aminobutyric acid betaine yields varied from 0.02% to 0.08% (mean 0.04%) and δ-aminovaleric acid betaine from 0.01% to 0.03% (mean 0.02%). The equivalent ranges for the collections from different places at different times of the year were γ-aminobutyric acid betaine 0.02% to 0.08%

Table 2: Betaine yields (%, dry wt) from *Ascophyllum nodosum* samples collected from Cliff Cottages Beach, Dale, Pembrokeshire, Wales, UK

Date of collection	gb	γ-abab	δ-avab	Total
December 1st, 2006	nd	0.08	0.04	0.12
December 26th, 2006	nd	0.10	0.05	0.15
February 1st, 2007	nd	0.10	0.07	0.17
March 1st, 2007	nd	0.14	0.04	0.18
March 30th, 2007	+	0.12	0.06	0.18
May 8th, 2007	0.01	0.10	0.04	0.15
June 5th, 2007	nd	0.05	0.03	0.08
July 2nd, 2007	nd	0.13	0.05	0.18
August 7th, 2007	nd	0.07	0.05	0.12
September 4th, 2007	nd	0.08	0.04	0.12
October 15th, 2007	nd	0.09	0.05	0.14
November 1st, 2008	+	0.06	0.04	0.10
January 21st, 2009	nd	0.09	0.05	0.14
February 17th, 2009	nd	0.13	0.07	0.20

gb = glycinebetaine; γ-abab = γ-aminobutyric acid betaine; δ-avab = δ-aminovaleric acid betaine; + = less than 0.01%; nd = not detected

(mean 0.05%) and δ-aminovaleric acid betaine 0.01% to 0.05% (mean 0.02%). However, the samples from Cliff Cottages Beach, Dale in Pembrokeshire (Table 2), which were collected at approximately monthly intervals, gave significantly higher betaine yields than those recorded in Table 1a and those of the Finavarra samples collected in May ($p < 0.001$). However, there was no clear cut seasonal variation in betaine yields from the Pembrokeshire collections [γ-aminobutyric acid betaine 0.06% to 0.14% (mean 0.10%) and δ-aminovaleric acid betaine 0.03% to 0.07% (mean 0.05%)]. However, further study to verify possible seasonal variation would be necessary. McKinnon *et al.* [10] found differences in betaine contents between 'olive' and 'brown' varieties of *A. nodosum* and also recorded seasonal variations in betaine yields.

The results presented in this paper indicate that, irrespective of either collection site or date of collection, the major betaines in each of the four investigated seaweed species remain constant. Also, as a generalization, there appeared to be little quantitative variation in the betaine contents of samples taken from different sites and at different times of the year. However, an exception appeared to be *A. nodosum*, samples from one collection area of which had significantly higher betaine contents than the other sites from where collections were made.

Experimental

Algal materials: The dates and places of collection of the various samples of *Ascophyllum nodosum* (L.) Le Jolis, *Fucus serratus* L. (family Fucaceae), *Laminaria digitata* (Huds.) Lamour. and *L. hyperborea* (Gunn.) Fosl., (family Laminariaceae) made from a wide variety of places and at different times of the year, are given in Table 1. To determine possible variations in betaine yields with the time of year, approximately monthly collections were made of *A. nodosum* from Cliff Cottages Beach, Dale, Pembrokeshire, Wales, UK. To determine variations in betaine yields from collections made of the same species at the same time of the year from the same area, 10 samples of each species were taken between May 23rd and 25th, 2009 from the Finavarra area of County Clare, Ireland When possible, each sample was made up of parts from at least 3 plants. Representative samples of each of the species are lodged in the Herbarium of the Hampshire County Council Museum Service, Winchester, Hampshire, UK (Index Herbariorum code HCMS; accession number Bi 2000. 16).

After collection, the algal material was dried in a circulating air oven at 60°C and powdered.

Extraction, purification and betaine estimation: Powdered samples of each collection (3-4 g, accurately weighed) were extracted in a Soxhlet extractor with 80% aqueous methanol for 5 h and the extracts purified using a column of cation exchange resin (Amberlite IR-120 H+ form), as reported previously [3a, 7]. The betaines present in the semi-purified extracts were identified by TLC [7] and ^{1}H NMR (D_2O; 400 MHz; Jeol Eclipe) [8]. The content of each of the major betaines present in the semi-purified extracts was estimated using a ^{1}H NMR spectroscopic assay procedure [9].

Statistical analysis: The data were analyzed for significant differences using the Kruskal-Wallis non-parametric test [13].

Acknowledgments – We thank the following for collection of samples: Dr V. Stiger-Pouvreau (Nelon, France), J. and L. Sawtell (Wembury and Guernsey), Dr A. M. Mortensen (Hella, Faroe Islands), Dr K. Sjøtun (Bergen, Norway), D. and J. Jackson (Scarborough), J. Fisher (September samples from the Isle of Man), Dr R. Ugarte (Canada), and Professor M. D. Guiry (some Irish samples). We are also grateful to Nigel Armstrong for the ^{1}H NMR spectra, and Abigail Wood for technical assistance. Thanks are also due to the Martin Ryan Institute, National University of Ireland, Galway, for use of the Marine Research Station at Finavarra, Co. Clare.

References

[1] (a) Blunden G. (**1991**) Agricultural uses of seaweeds and seaweed extracts. In *Seaweed Resources in Europe: Uses and Potential.* Guiry MD, Blunden G. (Eds). John Wiley & Sons, Chichester, UK. 65-81; (b) Khan W, Rayirath WP, Subramanian S, Mundaya NJ, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B. (**2009**). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation, **28***, 386 – 399.

[2] Blunden G, Rogers DJ, Barwell CJ. (**1984**) Biologically-active compounds from British marine algae. In *Natural Products and Drug Development*, Krogsgaard-Larsen P, Brϴgger-Cristensen S, Kofod H. (Eds), Munksgaard, Copenhagen, 179-190.

[3] (a) Blunden G, Gorden SM, Smith BE, Fletcher RL. (**1985**) Quaternary ammonium compounds in species of the Fucaceae (Phaeophyceae) from Britain. *British Phycological Journal*, ***20***, 105-108; (b) Blunden G, Gordon SM. (**1986**) Betaines and their sulphonio analogues in marine algae. In *Progress in Phycological Research*, Vol. 4, Round FE, Chapman DJ. (Eds), Biopress, Bristol, 39-80.

[4] (a) Blunden G, Jenkins T, Liu YW. (**1997**) Enhanced leaf chlorophyll levels in plants treated with seaweed extract. *Journal of Applied Phycology*, ***8***, 535-543; (b) Wu Y, Jenkins T, Blunden G, Whapham C, Hankins S. (**1997**) The role of betaines in alkaline extracts of *Ascophyllum nodosum* in the reduction of *Meloidogyne javanica* and *M. incognita* infestations of tomato plants. *Fundamental and Applied Nematology*, ***20***, 99-102.

[5] (a) Jayaraj J, Wan A, Rahman M, Punja ZK. (**2008**) Seaweed extract reduces foliar fungal diseases on carrot. *Crop Protection*, ***27***, 1360-1366; (b) Kraska T, Schönbeck F. (**1992**) Resistance induction in plants by trigonelline and possible mechanisms. In *Proceedings of the Third International Conference on Role of Formaldehyde in Biological Systems. Methylation and Demethylation Processes*. Tyihák E. (Ed.). Hungarian Biochemical Society, Budapest. 157-162; (c) Tyihák E, Steiner U, Schönbeck F. (**2002**) Time- and dose-dependent double immune response of plants to pathogens. In *Modern Fungicides and Antifungal Compounds III*, AgroConcept, Bonn, 187-196; (d) Tyihák E. (**2006**) Double immune response of plants to pathogens and its biochemical basis. In *Floriculture, Ornamental and Plant Biotechnology. Advances and Topical Issues, Vol. III.* Teixeira da Silva JA. (Ed.). Global Science Books, UK 380-387.

[6] Rayirath P, Benkel B, Hodges DM, Allan-Wojtas P, MacKinnon S, Critchley AT, Prithiviraj B. (**in press**) Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in *Arabidopsis thaliana. Planta*, DOI:10.1007/s00425-009-0920-8.

[7] Blunden G, El Barouni MM, Gordon SM, McLean WFH, Rogers DJ. (**1981**) Extraction, purification and characterisation of Dragendorff-positive compounds from some British marine algae. *Botanica Marina*, ***24***, 451-456.

[8] Blunden G, Gordon SM, Crabb TA, Roch OG, Rowan MG, Wood B. (**1986**) NMR spectra of betaines from marine algae. *Magnetic Resonance in Chemistry*, ***24***, 965-971.

[9] Blunden G, Cripps AL, Gordon SM, Mason TG, Turner CH. (**1986**) The characterization and quantitative estimation of betaines in commercial seaweed extracts. *Botanica Marina*, ***29***, 155-160.

[10] McKinnon SL, Hiltz D, Ugarte R, Craft CA. (**in press**) Improved method of analysis for betaines in *Ascophyllum nodosum* and its commercial extracts. *Journal of Applied Phycology*, DOI:10.1007/s10811-009-9483-0.

[11] (a) Blunden G, Patel AV, Armstrong N, Adrian Romero M, Meléndez P. (**2005**) Betaine distribution in Angiosperms. *Biochemical Systematics and Ecology*, ***33***, 904-920; (b) Ashihara H. (**2008**) Trigonelline (*N*-methylnicotinic acid) biosynthesis and its biological role in plants. *Natural Product Communications*, ***3***, 1423-1428.

[12] Blunden G, Gordon SM, Keysell GR. (**1982**) Lysine betaine and other quaternary ammonium compounds from British species of the Laminariales. *Journal of Natural Products.* ***45***, 449-452.

[13] Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. (**2007**) Genstat for Windows (10^{th} Ed.). VSN International, Hemel Hempstead, UK