

# LYSINE BETAINE AND OTHER QUATERNARY AMMONIUM COMPOUNDS FROM BRITISH SPECIES OF THE LAMINARIALES

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**ABSTRACT.**—The quaternary ammonium compounds extracted from the British species of Laminariales have been studied. Both laminine and lysine betaine, as their dioxalates, have been isolated from *Laminaria hyperborea* and characterized by tlc and by ir and nmr spectroscopy. Lysine betaine has not been isolated previously from a natural source. Tlc evidence supports the presence also of glycine betaine,  $\gamma$ -butyrobetaine and, in a spring collection, choline. The same compounds were detected by tlc in extracts of *L. digitata*, *L. ochroleuca* and *L. saccharina*. In *Saccorhiza polyschides*, *Alaria esculenta* and *Chorda filum*, glycine betaine was the major quaternary ammonium compound found.

Quaternary ammonium and other Dragendorff-positive compounds have been recorded for several marine algae. The compounds found and their distribution have been reviewed recently by us (1). In addition to the substances listed in that paper, taurine betaine (2-4), N,N-dimethyltaurine (2-6), choline-O-sulfate (6) and 1,3-dimethylhistidine (7) have been recorded for marine algal species. In this present communication we record the results of a study made of the Dragendorff-positive compounds detected in British species of the Laminariales.

## MATERIALS AND METHODS

**PLANT MATERIALS.**—The seaweeds used and their places and dates of collection were as follows: *Laminaria digitata* (Huds.) Lamour., Lulworth Cove, Dorset, April and September 1983; *L. hyperborea* (Gunn.) Fosl., Lulworth Cove, April and September 1980; *L. ochroleuca* Pyl., Scilly Isles, June 1980; *L. saccharina* (L.) Lamour., Kimmeridge, Dorset, May and September 1980; *Saccorhiza polyschides* (Lightf.) Batt., Lulworth Cove, September 1980; *Alaria esculenta* (L.) Grev., Scilly Isles, July 1980; and *Chorda filum* (L.) Stackh., Spithead, E. Solent, Hampshire, September 1980.

As soon as possible after collection, each species was dried in a circulating air oven at a temperature not exceeding 60° and powdered.

**EXTRACTION, PARTIAL PURIFICATION AND CHROMATOGRAPHIC EXAMINATION OF QUATERNARY AMMONIUM COMPOUNDS.**—Dried powdered alga was extracted with 80% aqueous methanol for 10 hr. The extract was concentrated under reduced pressure to remove the methanol, filtered to remove precipitated material and the aqueous filtrate partially purified by ion exchange chromatography (1). The resulting extract was studied by tlc on air-dried layers of either silica gel G (Merck), 250  $\mu$ m wet thickness, or cellulose (Whatman CC 41), 500  $\mu$ m wet thickness; the 9 development systems described earlier (1) were used.

When the presence of choline was indicated from the tlc results, the extract was examined by electrophoresis (1).

The quaternary ammonium compounds were detected on tlc and electrophoretic layers by spraying with Dragendorff's reagent, followed by overspraying with a saturated solution of sodium hydrogen sulfate (1) and by drying the layers in a current of cold air.

**ISOLATION OF QUATERNARY AMMONIUM COMPOUNDS.**—The individual quaternary ammonium compounds were separated by preparative tlc on silica gel H layers, 500  $\mu$ m thick, and double development in methanol-water (50:50 v/v). Each compound was eluted with 80% aqueous methanol, the extract filtered and evaporated to small volume under reduced pressure. If necessary, the extract was purified by passage through a column of anion exchange resin (1). The elute was concentrated to a small volume, and saturated aqueous oxalic acid solution was added to it. After mixing, the oxalate produced was precipitated with ethanol, removed by filtration, dried and recrystallized from aqueous ethanol. Each isolated compound was examined by ir and nmr spectroscopy. Ir spectra were obtained from potassium bromide discs on a Perkin Elmer 377 Grating Infra-Red Spectrophotometer, and nmr spectra were determined in deuterium oxide on a Bruker 270 MHz machine.

**SYNTHESIS OF LAMININE AND LYSINE BETAINE.**—Both laminine and lysine betaine, as their dioxalates, were synthesized from L-lysine by the methods described by Takemoto *et al.* (8); the copper complex procedure was used for the production of laminine.

## RESULTS AND DISCUSSION

*Laminaria hyperborea* was collected in September and extracted. To purify the extract, it was added to a column of cation exchange resin. The quaternary ammonium compounds eluted from the column were examined by tlc, and 5 Dragendorff-positive compounds were detected, each of which was separated by preparative tlc. The major component co-chromatographed, in all 9 systems used, with laminine (fig. 1a). The compound was isolated as its dioxalate, mp 124–125°, undepressed on admixture with laminine dioxalate. The isolated material had ir and nmr spectroscopic characters identical to those of laminine dioxalate. The nmr spectrum showed resonances at  $\delta$  1.47 (2H, m;  $\gamma$ -CH<sub>2</sub>),  $\delta$  1.84 (2H, m;  $\delta$ -CH<sub>2</sub>),  $\delta$  1.95 (2H, m;  $\beta$ -CH<sub>2</sub>),  $\delta$  3.04 (9H, s; N<sup>+</sup>-(CH<sub>3</sub>)<sub>3</sub>),  $\delta$  3.27 (2H, m;  $\epsilon$ -CH<sub>2</sub>) and  $\delta$  3.84 (1H, t,  $J \approx 6$  Hz;  $\alpha$ -CH). Irradiation of the  $\epsilon$ -CH<sub>2</sub> protons at  $\delta$  3.27 reduced the multiplet produced by the  $\delta$ -CH<sub>2</sub> protons at  $\delta$  1.84 to a triplet. The  $\alpha$ -CH and  $\beta$ - and  $\gamma$ -CH<sub>2</sub> resonances were assigned by comparison with the spectrum of lysine betaine (see below). The data obtained show the identity of the major Dragendorff-positive compound in *L. hyperborea* to be laminine, which has been reported previously for several other species of *Laminaria* (9, 10).

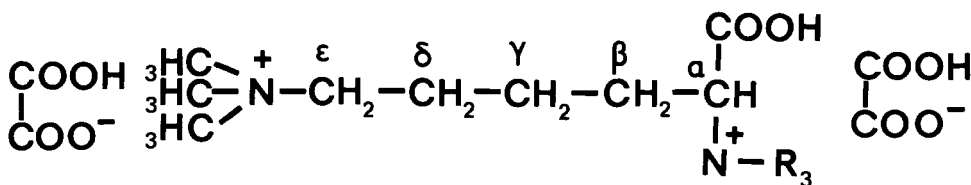


FIG. 1. Laminine dioxalate and lysine betaine dioxalate.

(a) R = H = laminine dioxalate

(b) R = CH<sub>3</sub> = lysine betaine dioxalate.

The second most abundant quaternary ammonium compound in *L. hyperborea*, when studied by tlc, produced a characteristic magenta color with Dragendorff's reagent and separated from all the reference compounds available to us that have been recorded previously for marine algae (1). The isolated compound, however, produced the same color with Dragendorff's reagent and, on tlc examination, did not separate from a synthetic sample of lysine betaine dioxalate (1-carboxy-N<sup>α</sup>, N<sup>α</sup>, N<sup>ε</sup>, N<sup>ε</sup>, N<sup>ε</sup>-hexamethyl-1,5-pentamethylene bisammonium dioxalate) (fig. 1b), synthesized originally by Takemoto *et al.* (8). We were unable to crystallize the compound from *L. hyperborea* as its hydrochloride but produced the dioxalate in crystalline form, mp greater than 360°. Both the ir and nmr spectra of synthetic lysine betaine dioxalate and the isolated compound were identical. The nmr spectrum showed resonances at  $\delta$  1.41 (2H, m;  $\gamma$ -CH<sub>2</sub>),  $\delta$  1.88 (2H, m;  $\delta$ -CH<sub>2</sub>),  $\delta$  1.97 (2H, m;  $\beta$ -CH<sub>2</sub>),  $\delta$  3.04 (9H, s;  $\epsilon$ -N<sup>+</sup>-(CH<sub>3</sub>)<sub>3</sub>),  $\delta$  3.12 (9H, s;  $\alpha$ -N<sup>+</sup>-(CH<sub>3</sub>)<sub>3</sub>),  $\delta$  3.27 (2H, t,  $J \approx 8$  Hz;  $\epsilon$ -CH<sub>2</sub>) and  $\delta$  3.65 (1H, m;  $\alpha$ -CH). Irradiation of the  $\beta$ -CH<sub>2</sub> protons at  $\delta$  1.97 revealed the  $\alpha$ -CH and  $\gamma$ -CH<sub>2</sub> signals; irradiation of the  $\epsilon$ -CH<sub>2</sub> protons at  $\delta$  3.27 enabled the assignment to be made of the  $\delta$ -CH<sub>2</sub> signal. The tlc, ir and nmr data prove that the compound isolated from *L. hyperborea* is lysine betaine. To our knowledge, this is the first time that lysine betaine has been isolated from a natural source.

The other 3 Dragendorff-positive compounds isolated were not obtained in sufficient amounts to fully characterize them. However, 2 compounds co-chromatographed in all 9 systems with either glycine betaine or  $\gamma$ -butyrobetaine. As a result, these identities have been given to the isolated compounds. The third component, present in very small amount, separated from all the reference materials available to us when studied by tlc.

The same quaternary ammonium compounds were detected by tlc in extracts of *L. hyperborea* collected in April, although the yields were considerably lower. However, in addition, a compound which had identical tlc and electrophoretic properties to choline was detected in extracts of the spring collection. No difference was observed in the compounds present or their relative amounts in extracts of new and old laminae collected in April. Similarly, no difference was detected between extracts of stipes and laminae collected in September.

Extracts of the other British species of Laminariales collected were examined by tlc for the presence of Dragendorff-positive materials. The results obtained are given in table 1. All the species studied yielded low quantities of quaternary ammonium compounds. The same substances were detected in all 4 species of *Laminaria*. As with *L. hyperborea*, choline was indicated in spring collections of *L. digitata* and *L. saccharina*. No collection of *L. ochroleuca* was made at that time.

TABLE 1. Dragendorff-positive compounds detected by tlc in extracts of British species of Laminariales.

Plant Material	Dragendorff-positive compounds detected					
	Glycine betaine	$\gamma$ -butyrobetaine	Laminine	Lysine betaine	Unknown A	Unknown B
Laminariaceae						
<i>Laminaria digitata</i> .....	+	+	++	+	±	-
<i>L. hyperborea</i> .....	+	+	++	+	±	-
<i>L. ochroleuca</i> .....	+	+	++	+	±	-
<i>L. saccharina</i> .....	+	+	++	+	±	-
<i>Saccorhiza polyschides</i> .	++	+	-	-	-	±
Alariaceae						
<i>Alaria esculenta</i> .....	++	-	+	-	-	-
Chordaceae						
<i>Chorda filum</i> .....	++	-	-	+	-	-

++ major component isolated, + detected, ± trace amount detected, - not detected.

Unlike the *Laminaria* species, in which laminine was the major Dragendorff-positive component, *Saccorhiza polyschides* yielded glycine betaine as its major quaternary ammonium compound; neither laminine nor lysine betaine was detected. A minor, unidentified component, different from that in the extracts of the *Laminaria* species, was recorded. Like *S. polyschides*, the major Dragendorff-positive compound in both *Alaria esculenta* and *Chorda filum* was glycine betaine. Laminine was detected also in *A. esculenta* and lysine betaine in *C. filum*.

The consistency in the distribution of quaternary ammonium compounds in the 4 *Laminaria* species studied and the differing occurrence of these compounds in the other genera of the Laminariales indicate that their presence or absence may be of taxonomic significance.

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#### LITERATURE CITED

1. G. Blunden, M. M. El Barouni, S. M. Gordon, W. F. H. McLean and D. J. Rogers, *Bot. Mar.*, **24**, 451 (1981).
2. G. Impellizzeri, S. Mangiafico, G. Oriente, M. Piattelli, S. Sciuto, E. Fattorusso, S. Magno, C. Santacroce and D. Sica, *Phytochemistry*, **14**, 1549 (1975).
3. V. Amico, G. Oriente, M. Piattelli, C. Tringali, E. Fattorusso, S. Magno, L. Mayol, C. Santacroce and D. Sica, *Biochem. Syst. Ecol.*, **4**, 143 (1976).

4. M. Freire and I. Ribas, *An. Quim.*, **71**, 418 (1975).
5. B. Lindberg, *Acta Chem. Scand.*, **9**, 1093 (1955).
6. B. Lindberg, *Acta Chem. Scand.*, **9**, 1323 (1955).
7. J. C. Madgwick and B. J. Ralph, *Bot. Mar.*, **15**, 205 (1972).
8. T. Takemoto, K. Daigo and N. Takagi, *J. Pharm. Soc. Japan*, **84**, 1180 (1964).
9. T. Takemoto, K. Daigo and N. Takagi, *J. Pharm. Soc. Japan*, **84**, 37 (1964).
10. T. Takemoto, K. Daigo and N. Takagi, *J. Pharm. Soc. Japan*, **85**, 37 (1965).