Carbohydrate Research, 246 (1993) 51–59
Elsevier Science Publishers B.V., Amsterdam

A new and rapid method for carrageenan identification by FT IR diffuse reflectance spectroscopy directly on dried, ground algal material

Thierry Chopin * and Ellen Whalen

Marine and Estuarine Research Group, Department of Biology, University of New Brunswick, P.O. Box 5050, Saint John, NB, E2L 4L5 (Canada)

(Received February 25th, 1993; accepted April 19th, 1993)

ABSTRACT

Carrageenans present in an alga can be identified rapidly by FT IR diffuse reflectance spectroscopy directly on only a few milligrams of dried, ground algal material, hence avoiding lengthy extraction procedures. Not only are the major types of carrageenans (such as κ-, λ-, and ι-carrageenans) detected, but so are smaller fractions (such as the precursors μ-, ν-, and o-carrageenans). Because the samples are minimally treated, the determined composition represents, as accurately as possible, the natural composition of the plants.

INTRODUCTION

Carrageenans are cell-wall sulfated polysaccharides produced by algae belonging to several families of the Rhodophyta and are widely used, predominantly in the food, pharmaceutical, and cosmetic industries[1]. Their repeating disaccharide backbone unit, β-(1 → 4)-D-galactopyranosyl-α-(1 → 3)-D-galactopyranosyl, may be substituted or modified in a variety of ways [β-(1 → 4)-D-galactopyranosyl replaced by β-(1 → 4)-3,6-anhydro-D-galactopyranosyl; degree and pattern of sulfation, methylation, or pyruvation] leading to the present recognition of three carrageenan families: κ, λ, and β[2]. These variations in composition are, in part, responsible for the different rheological properties and uses of carrageenans (gelling, thickening, stabilizing, binding, clarifying, etc., agents)[1]. The world market is, at the present time, based on three types of carrageenans: the κ- and ι-carrageenans (gelling agents), and the λ- carrageenan (nongelling agent)[3]. The other types of carrageenans, except β, are commonly found in minor amounts in many of the

* Corresponding author.

carrageenophytes, which are, consequently, now regarded as hybrids and no longer as producers of a sole type of carrageenan[2,4].

Generally, the types of carrageenans produced by an alga are identified after the carrageenans have been extracted from the cell wall of plants, filtered, concentrated, precipitated, separated, washed, dried, and finally ground. This is a long and tedious process, especially if using the carrageenan-selective hexadecyltrimethylammonium bromide (CTAB) precipitation method versus the nonselective alcohol precipitation method[5].

This paper describes a new and rapid method, using FT IR diffuse reflectance spectroscopy, to directly identify the types of carrageenans in an alga, with only a few milligrams of dried, ground material.

EXPERIMENTAL

Materials.—A sample of *Chondrus crispus* Stackhouse was collected in November 1992 at Maces Bay on the New Brunswick side of the Bay of Fundy, Canada. Plants were sorted into five classes, based on the number of dichotomies and frond length[6]. The largest plants of Class 5 (fronds with any number of dichotomies and a frond length greater than 10 cm) were selected and then sorted into three groups: (*1*) reproductive female gametophytes (known to produce predominantly κ-carrageenans[7]), (*2*) reproductive tetrasporophytes (known to produce predominantly λ-carrageenans[8]), and (*3*) vegetative fronds (which, on occasion, included difficult-to-discern mature, male gametophytes).

A sample of *Eucheuma denticulatum* (Burman) Collins and Hervey (also described as *E. spinosum*, and known to produce predominantly ι-carrageenans[9]) was kindly provided by the Marine Colloids Division of FMC Corporation (Rockland, ME, USA). It was collected in February 1992 at a seaweed farm in Tawi-Tawi, Southern Philippines.

Extraction and content of total carrageenans.—Samples of *C. crispus* (female gametophytes and tetrasporophytes) and of *E. denticulatum* were extracted, and the carrageenans were selectively precipitated with CTAB[10,11]. Coagula were dried in an oven for 72 h at 60°C, and then weighed to determine the content or yield [per cent dry weight (dw)].

Grinding.—Extracted carrageenans and oven-dried (72 h at 60°C) pieces of algae were each ground for 15 min to a fine powder with a Retsch Mixer Mill MM2. The different powders were then oven-dried for 24 h at 60°C.

FT IR spectroscopy.—Samples (35 mg) of ground, dried algal material, or carrageenans, were mixed with IR-grade KBr powder (350 mg) and further ground with mortar and pestle. The mixture was then used to fill to capacity a macro sampling cup (13 mm diameter, 2 mm depth) mounted on a Barnes/Spectra-Tech Collector Diffuse Reflectance Accessory (DRIFT). Spectra (128 scans) were collected with a Nicolet 520 P FT IR spectrometer at 2 cm^{-1} resolution.

TABLE I

Identification of the types of carrageenans according to the occurrence, or lack thereof, of certain peaks in their infrared spectra

Vibrations		Occurrence (+), lack thereof (−), of the peak according to the type of carrageenans				
Wavenumbers (cm^{-1})	Bond(s)/group(s)	κ	μ	ι	ν	λ
1240	S=O of sulfate esters	+	+	+	+	+
930	C–O of 3,6-anhydro-D-galactose	+	−	+	−	−
845	C–O–S of axial secondary sulfate on C-4 of galactose	+	+	+	+	−
830	C–O–S of equatorial secondary sulfate on C-2 of galactose	−	−	−	+	+
820	C–O–S of equatorial primary sulfate on C-6 of galactose	−	+	−	+	+
805	C–O–S of axial secondary sulfate on C-2 of 3,6-anhydro-D-galactose	−	−	+	−	−

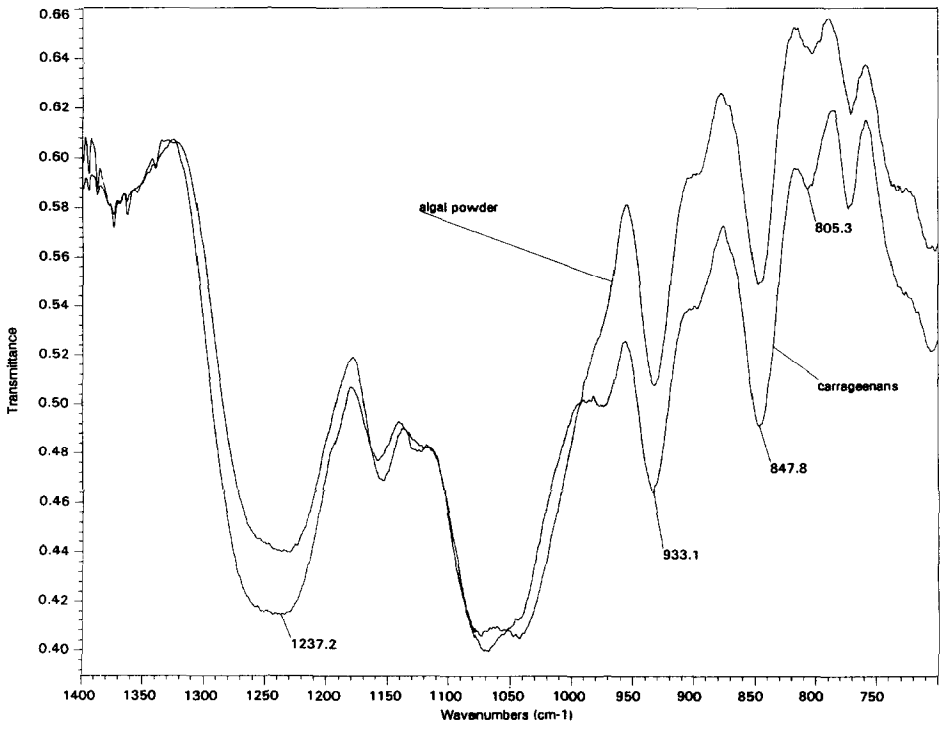

Fig. 1. FT IR spectra of untreated algal powder and carrageenans extracted from female gametophytes of *Chondrus crispus*.

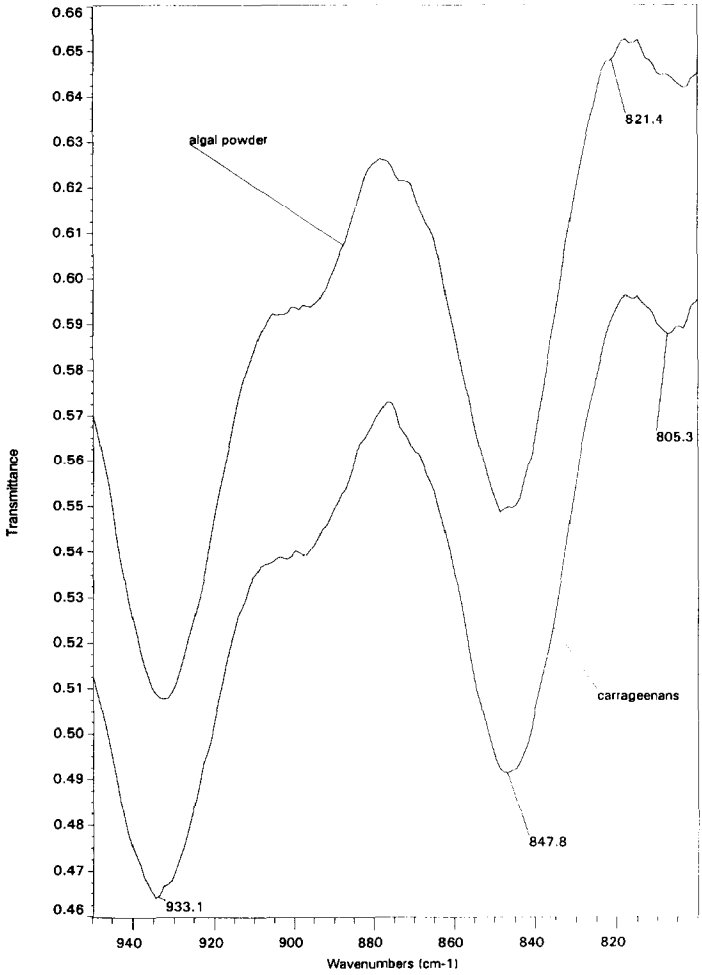

Fig. 2. Enlargement of the FT IR spectra presented in Fig. 1, between 950 and 800 cm^{-1}, revealing the presence of μ-carrageenan (shoulder at 821.4 cm^{-1}) in the untreated algal powder.

Reproducibility of the spectra was high, as grinding with a Retsch Mixer Mill MM2 and then with mortar and pestle ensured the obtention of a powder homogeneous in its particle size and mixing with KBr. In a preliminary study (data not shown), the ratio of dried, ground plants to KBr powder was varied from 1:10 to 3:7, the number of scans was increased as far up as 256, and the resolution decreased as far down as 0.5 cm^{-1}. The above conditions (1:10 ratio, 128 scans, and 2 cm^{-1} resolution) proved, however, to be the best.

RESULTS AND DISCUSSION

The three samples analyzed in this study had a relatively high carrageenan yield compared to other carrageenophytes[3]: 58.9% dw for the female gametophytes of

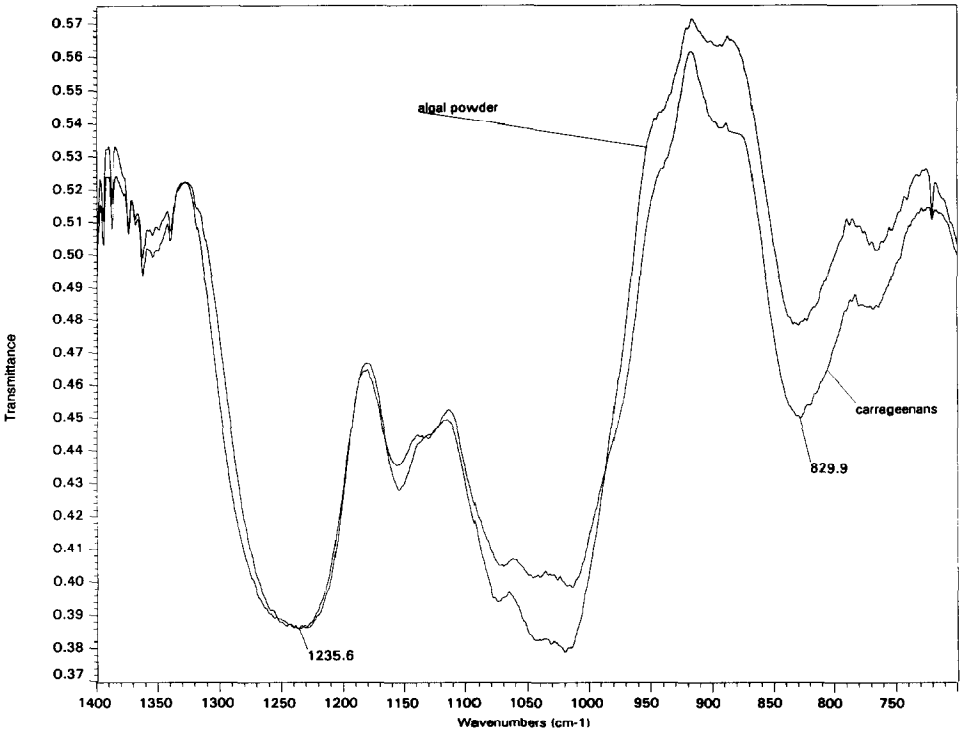

Fig. 3. FT IR spectra of untreated algal powder and carrageenans extracted from tetrasporophytes of *Chondrus crispus*.

C. crispus, 49.5% dw for the tetrasporophytes of *C. crispus*, and 61.6% dw for *E. denticulatum*.

When using infrared spectroscopy, the types of carrageenans present in an alga are identified by analysis of the occurrence, lack thereof, of certain peaks corresponding to the absorption bands of their structural elements (Table I)[12–16]. Fig. 1 shows the spectra of ground pieces of female gametophytes of *C. crispus*, without further treatment, and of the carrageenans extracted from them. Both spectra confirmed that these plants contain mainly carrageenans of the κ-type (peaks at 933.1 and 847.8 cm^{-1}). The small peak at 805.3 cm^{-1} indicates the presence of a minor amount of ι-carrageenan. Moreover, the spectrum of untreated algal powder shows a shoulder at 821.4 cm^{-1} (Fig. 2), revealing the presence of a small amount of μ-carrageenan (the precursor of κ-carrageenan[17,18]), which is not detectable in the spectrum of the extracted carrageenans.

Both spectra displayed in Fig. 3 show a broad peak at 829.9 cm^{-1} resulting from the association of the peaks generally described at 830 and 820 cm^{-1}, and characteristic of the λ-carrageenan produced by the tetrasporophytes of *C. crispus*.

The spectra of untreated algal powder and extracted carrageenans of *E. denticulatum* (Fig. 4) demonstrate that this species predominantly contains car-

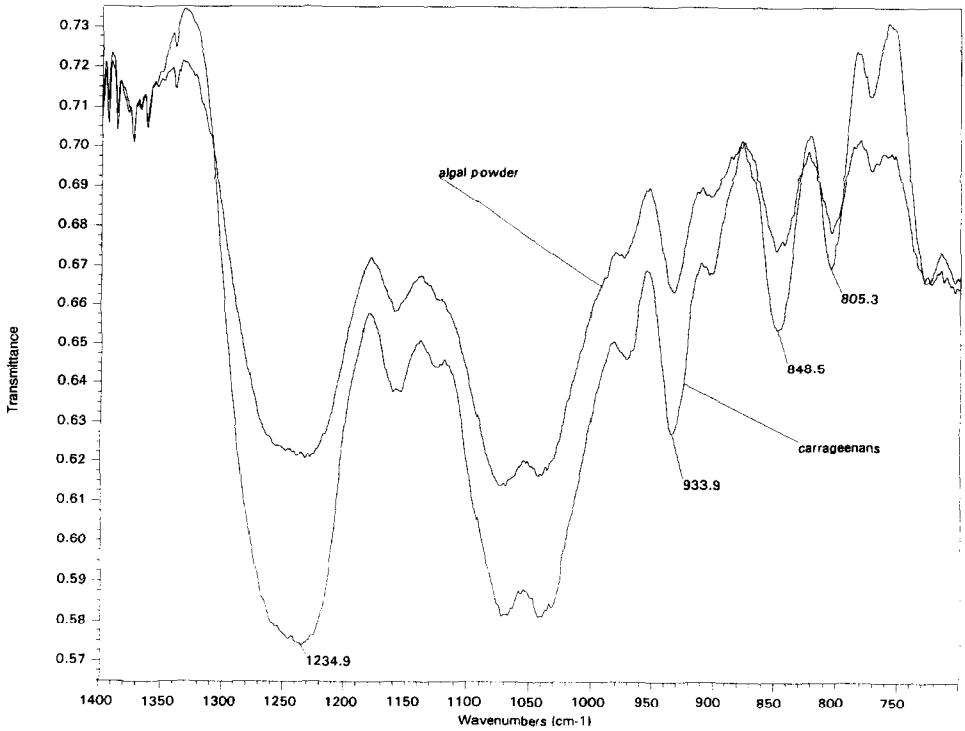

Fig. 4. FT IR spectra of algal powder and carrageenans extracted from *Eucheuma denticulatum*.

rageenans of the ι-type (peaks at 933.9, 848.5, and 805.3 cm^{-1}). We also report a peak at 904.6 cm^{-1} (Fig. 5), similar to the peak at 906 cm^{-1} mentioned by Mollion et al.[19] in their study of *E. denticulatum* from Madagascar; this signal is presently unassigned.

As previously described[11], observation of the region around 975 cm^{-1} (972.9 cm^{-1} on Fig. 5) gives a rapid means of determining if a sample contains only ι-carrageenan (distinct peak) or ι-carrageenan with a smaller proportion of deviant ι-carrageenan (shoulder). Consequently, the spectrum of the algal powder indicates that, when untreated, *E. denticulatum* contains not only ι-carrageenan, but also deviant ι-carrageenan (or ο-carrageenan, as recommended by Craigie[2]) as a minor component, thus confirming by FT IR spectroscopy what Mollion et al.[19] suggested, based on 3,6-anhydro-D-galactose measurements. The extraction technique could be seen as modifying the deviant ι-carrageenan fraction into ι-carrageenan, as the spectrum of the extracted carrageenans shows a peak at 972.9 cm^{-1}. Another interpretation, based on unpublished results about consecutive extractions until all carrageenans in *C. crispus* are exhaustively collected[20], would be to consider that the first extraction (the only one in this study) selectively recovers ι-carrageenan, while the following ones recover more and more deviant ι-carrageenan, due to a solubility difference between the two types of carrageenans.

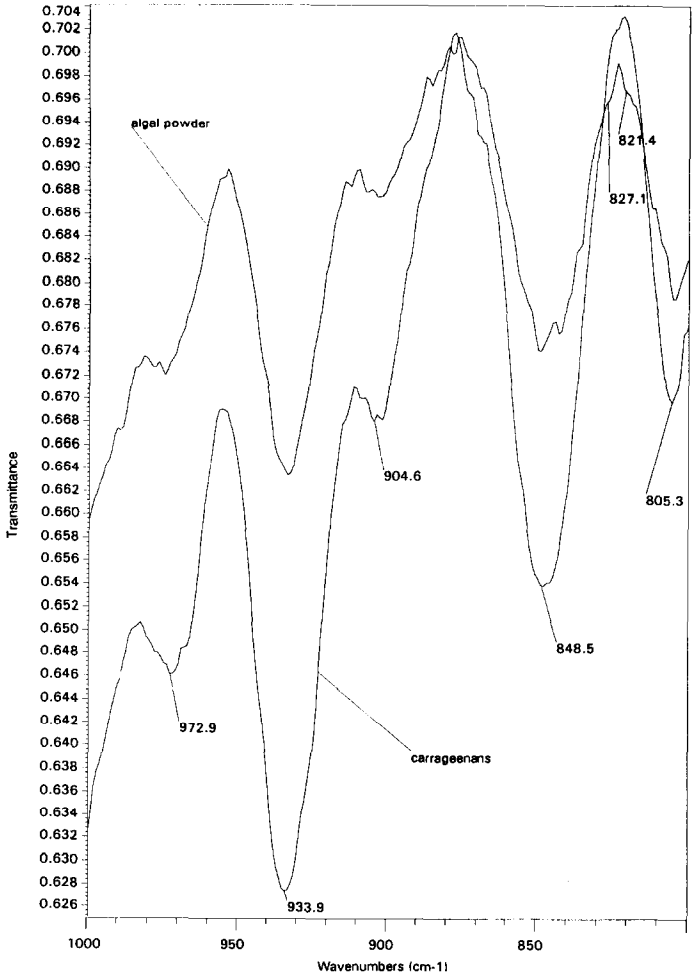

Fig. 5. Enlargement of the FT IR spectra presented in Fig. 4, between 1000 and 800 cm^{-1}, revealing in the untreated algal powder the presence of deviant ι-carrageenan (shoulder at 972.9 cm^{-1}), and ν-carrageenan (shoulders at 827.1 and 821.4 cm^{-1}).

The spectrum of the extracted carrageenans does not indicate the presence of any precursor, which could have led us to question, as did Mollion et al.[19], the occurrence of ν-carrageenan, the precursor of ι-carrageenan[17,18], in this species. The spectrum of untreated algal powder, however, shows two shoulders, at 827.1 and 821.4 cm^{-1}, which are characteristic of ν-carrageenan.

CONCLUSIONS

This study shows that in red algae in which cell-wall polysaccharides represent a significant percentage of the dry weight, pieces of dried, finely ground plants, mixed with KBr powder in the ratio 1:10, and analyzed by FT IR diffuse

reflectance spectroscopy, produce rapidly, without further treatment, spectra whose resolution procures much more information than that affordable by traditional dispersive IR techniques or by spectra of extracted carrageenans.

Small carrageenan fractions (such as the precursors μ-, ν-, and o-carrageenans) can be detected. Moreover, the carrageenans are identified on samples which have been minimally treated (only drying and grinding), which allows the most accurate determination of the natural composition of the different types of carrageenans, whereas extractive techniques, by the unavoidable selectivity and fractioning they introduce, yield compositions not necessarily reflective of the natural and complete composition.

The FT IR diffuse reflectance technique is, obviously, a much faster method than the classical preparation of films, or KBr pellets, with carrageenans recovered after lengthy extraction procedures. The fact that the types of carrageenans can be directly identified with only a few milligrams of dried, ground algal material should make this technique interesting to both industrial and academic scientists, as it allows rapid screening of algae of potential industrial interest, further investigation of the algal cell-wall chemistry, and chemo-taxonomical problems encountered in the different classes of red algae to be addressed using small amounts of dried material, originating either from small field or herbarium collections.

ACKNOWLEDGMENTS

This work was supported by NSERC grants OGP46376, EQP92706, and EQP121606 to T. Chopin. We thank Mr. Gordon Guist (Marine Colloids Division of FMC Corporation) for kindly providing the *E. denticulatum* sample and Mr. Patrick Savory [Nicolet Instrument Corp. (Canada)] for helpful discussions.

REFERENCES

1 T. Chopin, *Can. Tech. Rep. Fish. Aquat. Sci.*, 1514 (1986) 1–69.
2 J.S. Craigie, in K.M. Cole and R.G. Sheath (Eds.), *Biology of the Red Algae*, Cambridge University Press, Cambridge, 1990, pp 221–257.
3 R. Perez, R. Kaas, F. Campello, S. Arbault, and O. Barbaroux, *La Culture des Algues Marines dans le Monde*, IFREMER, Brest, 1992.
4 C. Bodeau-Bellion, *Physiol. Vég.*, 21 (1983) 785–793.
5 T. Chopin, M.D. Hanisak, and F.E. Koehn, *Bot. Mar.*, 34 (1991) 369–373.
6 T. Chopin, J.D. Pringle, and R.E. Semple, *Can. J. Fish. Aquat. Sci.*, 49 (1992) 349–357.
7 T. Chopin, C. Bodeau-Bellion, J.-Y. Floc'h, E. Guittet, and J.-Y. Lallemand, *Hydrobiologia*, 151/152 (1987) 535–539.
8 E.L. McCandless, J.S. Craigie, and J.A. Walter, *Planta*, 112 (1973) 201–212.
9 C. Bellion, G.K. Hamer, and W. Yaphe, *Proc. Int. Seaweed Symp.*, 10 (1981) 379–384.
10 J.S. Craigie and C. Leigh, in J.A. Hellebust and J.S. Craigie (Eds.), *Handbook of Phycological Methods, Physiological and Biochemical Methods*, Cambridge University Press, Cambridge, 1978, pp 109–131.
11 T. Chopin, M.D. Hanisak, F.E. Koehn, J. Mollion, and S. Moreau, *J. Appl. Phycol.*, 2 (1990) 3–16.
12 D.J. Stancioff and N.F. Stanley, *Proc. Int. Seaweed Symp.*, 6 (1969) 595–609.

13 N.F. Stanley, U.S. Patent 3 094 517 (1963).
14 A.G. Lloyd, K.S. Dodgson, R.G. Price, and F.A. Rose, *Biochim. Biophys. Acta*, 46 (1961) 108–115.
15 A.G. Lloyd, and K.S. Dodgson, *Biochim. Biophys. Acta*, 46 (1961) 116–120.
16 N.S. Anderson, T.C.S. Dolan, A. Penman, D.A. Rees, G.P. Mueller, D.J. Stancioff, and N.F. Stanley, *J. Chem. Soc. (C)*, (1968) 602–606.
17 C.J. Lawson and D.A. Rees, *Nature*, 227 (1970) 392–393.
18 K.F. Wong and J.S. Craigie, *Plant Physiol.*, 61 (1978) 663–666.
19 J. Mollion, M. Andriantsiferana, and M. Sekkal, *Hydrobiologia*, 204/205 (1990) 655–659.
20 T. Chopin, *Variations saisonnières de la nutrition phosphorée, des carraghénanes et de la croissance chez deux formes de l'algue rouge Chondrus crispus Stackhouse*, Doctoral thesis, University of Brest, 1985.