The Chemical Structure of Agar from *Gracilaria compressa* (C. Agardh) Greville, *G. cervicornis* (Turner) J. Agardh, *G. damaecornis* J. Agardh and *G. domingensis* Sonder ex Kützinger (Gigartinales, Rhodophyta)

M. Lahaye¹ and W. Yaphe²

McGill University, Department of Microbiology and Immunology, 3775 University Street, H3A 2B4 Montreal, Canada

(Accepted 24 March 1989)

Abstract

The chemical structure of agar fractions obtained from *Gracilaria compressa*, *G. cervicornis*, *G. damaecornis* and *G. domingensis* with a sequential solvent extraction has been determined by ¹³C-NMR spectroscopy. *Gracilaria compressa* agar consists of agarobiose, pyruvated agarobiose and a low concentration of 6-O-methylated agarobiose repeating units; *G. cervicornis* and *G. domingensis* agars are composed of 6-O-methylated agarobiose, agarobiose-6-sulfate, agarobiose, and a repeating unit with 4-O-methyl- α -L-galactose; finally, *G. damaecornis* agar is made of the same repeating units of the latter agars plus agarobiose-4-sulfate. From these four algae, a fraction enriched with pyruvated or sulfated repeating units was obtained in water at room temperature. An extract essentially composed of only 6-O-methylated agarobiose repeating units was obtained from the *Gracilaria* species with boiling 80% ethanol. The concentration of the methylated repeating unit decreased in the subsequent boiling in 60% and 40% ethanol extracts and in the hot water extract (121 °C), whereas charged repeating units were present in these agar fractions. Floridean starch contaminated the hot water extract.

The combination of sequential solvent extraction and ¹³C-NMR represents a convenient approach in determining the chemical heterogeneity of agar.

Introduction

Marine red algae belonging to the genus *Gracilaria* represent an important potential source for the industrial production of agar and agarose (McLachlan 1985). Although the rheological properties of most *Gracilaria* agar gels are usually far from those required for biotechnological applications (electrophoresis, chromatographies, ...), they are generally suitable for the food industry. In fact, the rheological properties reflect the chemical variations of the basic repeating disaccharide, agarobiose (Araki 1966, Rees 1969 and Fig. 1), that compose the family of polysaccharides in agar (Duckworth and Yaphe 1971, Izumi 1970,

Lahaye *et al.* 1986) and probably also the molecular weight of the different agarose molecules in agar (Rochas and Lahaye 1989 a).

Species, strain, and growth conditions of *Gracilaria* affect the chemical heterogeneity of agar (Lahaye and Yaphe 1988 and references herein) and thus, these important factors have to be taken into account when harvesting algae for the production of commercially suitable agar. Different methods are available for assessing the chemical structure of agars but probably, nuclear magnetic resonance (NMR) spectroscopy is the fastest and most reliable one. Recently, solid-state (CP-MAS) ¹³C-NMR spectroscopy has been used successfully to distinguish between agar and carrageenan structures as well as some of their substituent groups in dry pieces of seaweeds (Rochas and Lahaye 1989 b). In this paper, the chemical structure of

¹ Present address: INRA-LBTG, B. P. 527 rue de la Géraudière, 44026 Nantes Cedex 03, France

² Deceased, May 1986

Fig. 1. Structure of the agarobiose and substituted agarobiose repeating units:

- A. agarobiose: $R_1 = R_2 = H$; A: 3,6-anhydro- α -L-galactopyranose, G: β -D-galactopyranose.
 agarobiose-6-sulfate: $R_1 = SO_3$, $R_2 = H$; A⁺: 3,6-anhydro- α -L-galactopyranose, G⁺: β -D-galactopyranose-6-sulfate.
 agarobiose-4-sulfate: $R_1 = H$, $R_2 = SO_3$; A^o: 3,6-anhydro- α -L-galactopyranose, G^o: β -D-galactopyranose-4-sulfate.
 6-O-methyl agarobiose: $R_1 = CH_3$, $R_2 = H$; G': 6-O-methyl- β -D-galactopyranose.
 proposed branched repeating unit (Hirase *et al.* 1983); $R_1 = 4$ -O-methyl- α -L-galactopyranose, $R_2 = H$; B: 4-O-methyl- α -L-galactopyranose.
 B. pyruvated agarobiose; G⁻: (4,6,carboxyethylidene)- β -D-galactopyranose, A⁻: 3,6-anhydro- α -L-galactopyranose.

charged agars extracted from *Gracilaria compressa* (C. agardh) Greville, *G. cervicornis* (Turner) J. Agardh, *G. damaecornis* J. Agardh, and *G. domingensis* Sonder *ex* Kützinger previously reported by Duckworth *et al.* (1971), were re-investigated using the sequential solvent extraction described by Lahaye *et al.* (1986) and ^{13}C -NMR spectroscopy. These methods permitted the isolation and characterization of novel agar fractions. The fine chemical structure of the substituted agarobiose repeating units encountered in these fractions is described in more detail elsewhere (Lahaye *et al.* 1989).

Material and Methods

Gracilaria cervicornis (Turner) J. Agardh (referred to as *G. ferox* by Hong *et al.* 1969 and Duckworth *et al.* 1971), *G. damaecornis* J. Agardh, *G. domingensis* Sonder *ex* Kützinger, and *G. compressa* (C. Agardh) Greville samples were those investigated by Hong *et al.* (1969) and Duckworth *et al.* (1971). Agar extraction from these algae followed the procedure described by Lahaye *et al.* (1986) with the following modifications: the dry algal pieces (10 g) were left at

room temperature with stirring for 4 hours or overnight in 95% ethanol (300 ml), the pigmented extract was discarded and the algal pieces extracted with boiling 95% ethanol as described by Lahaye *et al.* (1986); the boiling 20% ethanol and hot water (100 °C) extracts were omitted. ^{13}C -NMR spectroscopy was carried out using a Bruker WH 400 operating at 100.6 MHz. Spectra of about 5% polysaccharide solution in D₂O were recorded at 80 °C with a spectral width of 10–18 KHz and relaxation delay of 0.5 to 0.7 s. Carbon chemical shifts were measured in parts per million relative to internal dimethyl sulfide and converted to values related to tetramethylsilane (conversion constant: 39.6).

Results and Discussion

The yields of agar extracted with cold water (22 °C), boiling 80, 60, and 40% ethanol, and hot water (121 °C) are depicted in Figure 2. The total recovery of polymers on the basis of algal dry weight was 42.3, 37.2, 39.2, and 41.5% for *Gracilaria cervicornis*, *G. damaecornis*, *G. domingensis*, and *G. compressa*, respectively. Major fractions of agar were recovered with boiling 60, 40% ethanol and hot water (121 °C) (Fig. 2) except for *G. domingensis* for which the yield of the cold water extract was greater than that of the 40% ethanol extract (Fig. 2C).

Fig. 2. Yields of agar extracted from A, *Gracilaria cervicornis*, B, *G. damaecornis*, C, *G. domingensis*, D, *G. compressa* with water at 22 °C (CX), boiling 80, 60, 40% ethanol-water mixtures, and water at 121 °C (A). The yield in each extract represents a percentage of the dry weight of total agar extracted from the alga.

Fig. 3. ^{13}C -NMR spectrum of agar fractions extracted from *G. cervicornis* with water at 22 °C (CX) (1960 transients), boiling 80, 60, 40% ethanol-water mixture (779, 1116, 1132 transients, respectively) and water at 121 °C (A, 2519 transients). The letters represent carbons in the agarobiose or substituted agarobiose depicted in Figure 1; C refers to carbons in starch; 4- and 6-O-CH₃ refer to the methyl carbon in 4-O-methyl- α -L-galactose and 6-O-methyl- β -D-galactose, respectively.

The ^{13}C -NMR spectra of all fractions extracted from these algae confirmed the agar-type structure of the polysaccharides and demonstrated the presence of peculiarly substituted agarobiose repeating units.

Gracilaria cervicornis cold water extract showed a spectrum typical of agar essentially composed of repeating units containing D-galactose-6-sulfate [G^+1 : 102.6, 102.4; G^+4 : 68.4; G^+5 : 73.0; G^+6 : 67.4; A^+4 : 77.8, 77.6 ppm; Fig. 3 (CX); Usov *et al.* 1983] with split signals for G^+1 and A^+4 probably resulting from a non-uniform distribution of the sulfated repeating units in the polymers. Signal splittings have already been observed for agar with repeating units containing L-galactose-6-sulfate replacing 3,6-anhydrogalactose and believed to be the biological precursor to the agarobiose repeating unit (Lahaye *et al.* 1988 a, Morrice *et al.* 1983, Rees 1961). Small signals were observed for agarobiose (A, G, Usov *et al.* 1980, Rochas *et al.* 1986), and for 6-O-methylated agarobiose (G' , Nicolaisen *et al.* 1980). But, in contrast to the previously reported composition of cold water soluble agar (Lahaye and Yaphe 1988, Lahaye *et al.* 1986), no signal was detected for the biological precursor disaccharide unit.

The 80% ethanol extract agar from *G. cervicornis* had a quite different spectrum from that of the preceding fraction since it was typical of polymers composed essentially of 6-O-methylated agarobiose [Fig. 3 (80), G' , O- CH_3]. Small signals at 98.6, 70.8, and 61.9 ppm were attributed to the 4-O-methyl- α -L-galactose (Lahaye *et al.* 1988) known to be present in this agar (Hong *et al.* 1969, Duckworth *et al.* 1971, Bird *et al.* 1987) and probably occurring as a branch as proposed for *Laurencia* agar (Hirase *et al.* 1983). No signal was detected for unsubstituted agarobiose repeating units. The spectrum of the 60%, 40% ethanol and hot water extracts from that alga showed signals attributed to agar essentially composed of a combination of four types of repeating units: 6-O-methylated agarobiose, agarobiose containing D-galactose-6-sulfate, unsubstituted agarobiose, and a minor content of repeating unit containing 4-O-methyl- α -L-galactose [Fig. 3, (60, 40, 0)]. These fractions differed in the concentration of 6-O-methylated agarobiose, the highest signal intensity associated with this substituent being observed on the spectrum of the 60% ethanol extract. Signals for floridean starch were also observed (Fig. 3, C; Dais and Perlin 1982, Lahaye *et al.* 1986) and this polysaccharide accounted for about 51% of the hot

water extract, as determined from the integral of the anomeric signals, C1 (100.4, 100.2 ppm), G1 (102.5 ppm) and A1 (98.4 ppm). Other small signals, barely exceeding the noise level on these spectra, remain unassigned.

The ^{13}C -NMR spectra of agar fractions extracted from *G. domingensis* were similar to those of *G. cervicornis* (data not shown). Small signals for repeating units containing D-galactose-6-sulfate were observed on the spectrum of the 80% ethanol extract. Floridean starch accounted for about 30% of the hot water extract.

Another peculiar substituted agarose was that from *G. damaecornis* (Fig. 4). The spectrum of the cold water extract [Fig. 4 (CX)] showed major signals typical of polymers composed of agarobiose containing D-galactose-6-sulfate (G^+ , A^+) and agarobiose containing D-galactose-4-sulfate (A^+1 : 96.8, G^+2 : 71.0, G^+3 : 80.0, G^+4 : 77.0, G^+5 : 75.0, G^+6 : 61.3 ppm, Lahaye *et al.* 1988 a). Again, no signal was detected for the biological precursor to the agarobiose repeating unit.

In contrast, the 80% ethanol extract [Fig. 4 (80)] showed major NMR signals typical of 6-O-methylated agarobiose repeating units (G' , O- CH_3) and small signals at 102.6, 77.6, 73.0, 68.4, and 67.4 ppm attributed, respectively, to G^+1 , A^+4 , G^+5 , and G^+6 of agarobiose repeating units containing D-galactose-6-sulfate. No signal was detected for agarobiose repeating unit.

The 60%, 40% ethanol and hot water extracts [Fig. 4 (60, 40, 0)] showed complex NMR spectra. Signals were attributed to agar composed of repeating units containing D-galactose-6-sulfate, 6-O-methyl-D-galactose, D-galactose-4-sulfate, and 4-O-methyl- α -L-galactose. As for *G. cervicornis*, these fractions differed in their content of 6-O-methylated agarobiose. Signals for floridean starch were observed and this reserve polysaccharide accounted for about 19% of the hot water extract as roughly estimated from the integral of the anomeric signals.

Finally, the ^{13}C -NMR spectra of *G. compressa* agar fractions demonstrated the presence of pyruvate group on agarobiose repeating units. The spectrum of the cold water extract [Fig. 5 (CX)] showed typical signals associated with agar composed of agarobiose (A, G) and pyruvated agarobiose repeating units (A^-2 : 70.0, A^-4 : 77.5, G^-1 : 102.2, G^-3 : 79.5, G^-4 :

Fig. 4. ^{13}C -NMR spectrum of agar fractions extracted from *G. damaecornis* with water at 22 °C (CX) (1200 transients), boiling 80, 60, 40% ethanol-water mixture (1159, 800, 1721 transients, respectively) and water at 121 °C (A, 2565 transients). The letters represent carbons in the agarobiose or substituted agarobiose depicted in Figure 1; C refers to carbons in starch; 4- and 6-O- CH_3 refer to the methyl carbon in 4-O-methyl- α -L-galactose and 6-O-methyl- β -D-galactose, respectively.

71.6, G⁻5: 66.7, G⁻6: 65.3, C-CH₃: 25.7, COOH: 176.3 ppm, Lahaye *et al.* 1989). The relaxation time of the quaternary carbon of the pyruvate ketal was probably too long to give a signal under the spectral conditions used. No signal was detected for a repeating unit containing L-galactose-6-sulfate.

The 80% ethanol extract was determined from its ¹³C-NMR spectrum to be composed essentially of agarobiose and 6-O-methylated agarobiose repeating units [Fig. 5 (80), G⁻]. The signal for the methyl group of the methylated disaccharide repeating unit was not observed probably due to the spectral acquisition conditions.

The 60% and 40% ethanol extracts [Fig. 5 (60, 40)] showed major NMR signals attributed to agar polymers constituted of agarobiose and pyruvated agarobiose repeating units. Methylated agarobiose repeating units were not detected.

The ¹³C-NMR spectrum of the hot water extract [Fig. 5 (A)] showed signals demonstrating the presence in this fraction of a combination of agar and floridean starch. The latter accounted for 19% of the fraction, as estimated from the integral of the anomeric signals. The agar was mainly constituted of agarobiose and a low concentration of pyruvated agarobiose. The ratio of agarobiose to pyruvated agarobiose estimated from the integral of G5 and G⁻5 was lower than that of the other fractions (except the 80% ethanol extract). It was of 1:0.1 as compared to 1:0.3 in fraction 40, 1:0.4 in fraction 60, and 1:0.7 in fraction CX. The observation of agar having a low degree of pyruvation agrees with the results of Duckworth and Yaphe (1971) and Young *et al.* (1971) that demonstrated that pyruvation occurs on a peculiar family of agar in the alga. The biological significance of the pyruvated agarobiose distribution in *G. compressa* agar remains to be established. Other small signals barely exceeding the noise level on the spectrum remain unassigned.

Sequential extraction of agar from algae has been interpreted to reflect the solubility and association of polysaccharides in the algal cell-wall (Lahaye *et al.* 1986) and is not directly related to the weight-average molecular weight of the molecules (Rochas and Lahaye 1989 a). Thus, high concentrations of methoxyl and 3,6-anhydrogalactose in agar increase the hydrophobic properties of the molecules, allowing for their solubility in hot solutions of alcohol (40–80% boiling ethanol). In contrast, substitution of agarobiose with

alkali-stable charged groups increases hydrophilic properties with concomitant solubility of agar in polar solvents (rich in water). Furthermore, the replacement of anhydrogalactose by L-galactose-6-sulfate in the agarobiose introduces irregularity into the agarose helical structure (Arnott *et al.* 1974) and decrease the associations between polymers. In addition, the temperature at which agaroses are soluble is related to the conformation of the molecules and thus, to their non-covalent association between each other like in a gel. However, covalent cross-linkages between agaroses and other cell-wall polymers are suspected and are believed to modify the solubility of these molecules in requiring harsh conditions (water at 121 °C) for their extraction (Lahaye *et al.* 1988). The present results confirm that any high concentration of substituent groups disrupting the agarose helical conformation, and thus aggregation, such as sulfate and pyruvate, increases the solubility of the molecules, particularly in water at room temperature.

The present extraction method allowed for the solubilization of novel agar fractions such as highly sulfated agar containing D-galactose-4-sulfate and/or D-galactose-6-sulfate such as the cold water extract from *G. cervicornis*, *G. damaecornis*, and *G. domingensis*. The sulfated galactose residues were estimated from the integral of ¹³C-NMR signals to represent 60–90% of the D-galactose residues in these agars (about 16–25% sulfate on a weight basis). Although these values are comparable to those obtained from *Gloio-peltis* agar (Hirase and Watanabe 1971, Penman and Rees 1973), this is the first report of highly sulfated (alkali-stable) agar extracted from *Gracilaria* species.

Similarly, a highly pyruvated agar was obtained in the cold water extract from *G. compressa*. The concentration of pyruvated agarobiose in this agar (44%), estimated from the integrals of ¹³C-NMR signals, is approximately equivalent to 3 times (9.4% pyruvic acid on a weight basis) the concentration (2.8% on a weight basis) reported by Duckworth *et al.* (1971) and Young *et al.* (1971) from native *G. compressa* agar.

Several attempts have been made to correlate chemical structure to taxonomy of algae (Gretz *et al.* 1986 and references herein, Bird *et al.* 1987). The presence of D-galactose-6-sulfate in agar extracted from *G. cervicornis* and *G. domingensis*, the presence of pyruvated agarobiose repeat unit in *G. compressa* agar, and particularly, the regular distribution of galactose-

Fig. 5. ¹³C-NMR spectrum of agar fractions extracted from *G. compressa* with water at 22 °C (CX) (793 transients), boiling 80, 60, 40% ethanol-water mixture (2080, 1397, 1705 transients, respectively) and water at 121 °C (A, 2120 transients). The letters represent carbons in the agarobiose or substituted agarobiose depicted in Figure 1; C refers to carbons in starch.

4-sulfate in *G. damaecornis* agar (on average 4 repeating units contains D-galactose-4-sulfate per 10 repeating units containing D-galactose-6-sulfate or 6-O-methyl-galactose), may be characteristic of these species. It should also be noted that it is the first report of D-galactose-4-sulfate occurring as a major sugar in agar.

Observation of ^{13}C -NMR signals in spectra of agar fractions from *G. servicornis*, *G. damaecornis*, and *G. domingensis* attributed to 4-O-methyl- α -L-galactose occurring probably as a branch of the agarobiose repeating unit is in agreement with the thin layer chromatographic results of Duckworth *et al.* (1971) and with the pyrolysis-mass spectrometry data of Bird *et al.* (1987). Methylation is known to increase the gelling temperature of agars (Guiseley 1970); further work is now needed to determine the physico-chemical and rheological effects of this masking group in agar.

Physiological conditions are known to affect the growth rate of the algae and also the concentration of certain chemical structure in *Gracilaria* agar (Lahaye and Yaphe 1988). The present analyses were carried out on single samples and thus did not reflect the possible changes in concentration of substituent groups. A particularly important masked structure in agar which has not been observed here, is L-galactose-

6-sulfate since it has been proposed to be the biological precursor to the 3,6-anhydrogalactose residue (Rees 1961) and most probably reflects the growth status of the algae.

In conclusion, *Gracilaria* agar consists of a variety of related hybrid molecules varying in the extent and types of substituted repeating units. The chemical heterogeneity of these polysaccharides is conveniently determined using the combination of sequential solvent extraction and ^{13}C -NMR spectroscopy. The latter extraction procedure should prove useful together with anion exchange chromatography in isolating particular molecules to be used as models in the study of the physical and rheological properties of agarose.

Acknowledgements

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. The authors thank Dr Phan Viet and Ms. Bilo-deau for technical assistance and for use of the NMR facilities at the Laboratoire de Résonance Magnétique Nucléaire à Haut Champ, Université de Montréal, Canada; and Dr Rochas for the helpful criticism of the manuscript.

References

- Araki, C. 1966. Some recent studies on the polysaccharides of agarophytes. *Proc. Int. Seaweed Symp.* 5: 3–17.
- Arnott, S. A., A. Fulmer, W. E. Scott, I. C. M. Dea, R. Moorhouse and D. A. Rees. 1974. Agarose double helix and its function in agarose gel structure. *J. Mol. Biol.* 90: 269–284.
- Bird, C. J., R. J. Helleur, E. R. Hayes and J. McLachlan. 1987. Analytical pyrolysis as a taxonomic tool in *Gracilaria* (Rhodophyta: Gigartinales). *Proc. Int. Seaweed Symp.* 12: 207–212.
- Dais, P. and A. S. Perlin. 1982. High field, ^{13}C -NMR spectroscopy of β -glucans, amylopectin, and glycogen. *Carbohydr. Res.* 100: 103–116.
- Duckworth, M., K. C. Hong and W. Yaphe. 1971. The agar polysaccharides of *Gracilaria* species. *Carbohydr. Res.* 18: 1–19.
- Duckworth, M. and W. Yaphe. 1971. The structure of agar. Part 1: Fractionation of a complex mixture of polysaccharides. *Carbohydr. Res.* 16: 189–197.
- Gretz, M. R., J. M. Aronson and M. R. Sommerfeld. 1986. Cell wall composition of the conchocelis phases of *Bangia atropurpurea* and *Porphyra leucosticta* (Rhodophyta). *Bot. Mar.* 29: 91–96.
- Guiseley, K. B. 1970. The relationship between methoxyl content and gelling temperature of agarose. *Carbohydr. Res.* 13: 247–256.
- Hirase, S. and K. Watanabe. 1971. Fractionation and structural investigation of funoran. *Proc. Int. Seaweed Symp.* 7: 451–454.
- Hirase, S., K. Watanabe, R. Tanako and J. Tamura. 1983. Structural features of the sulfated polysaccharides isolated from the red seaweed *Laurencia undulata*. *Abstr. Int. Seaweed Symp., 11th*, Inst. of Oceanology, Academia Sinica, Qingdao, People's Republic of China, p. 93.
- Hong, K. C., M. E. Goldstein and W. Yaphe. 1969. A chemical and enzymatic analysis of the polysaccharides from *Gracilaria*. *Proc. Int. Seaweed Symp.* 6: 473–482.
- Izumi, K. 1970. A new method for fractionation of agar. *Agr. Biol. Chem.* 34: 1739–1740.
- Lahaye, M., J. F. Revol, C. Rochas, J. McLachlan and W. Yaphe. 1988. The chemical structure of *Gracilaria crassissima* (P. et H. Crouan in Schramm et Mazé) P. et H. Crouan in Schramm et Mazé and *G. tikvahiae* McLachlan (Gigartinales, Rhodophyta) cell wall polysaccharides. *Bot. Mar.* 31: 491–501.
- Lahaye, M., C. Rochas and W. Yaphe. 1986. A new procedure for determining the heterogeneity of agar polymers in the cell walls of *Gracilaria* spp. (Gracilariaceae, Rhodophyta). *Can. J. Bot.* 64: 579–585.
- Lahaye, M. and W. Yaphe. 1988. Effects of seasons on the chemical structure and gel strength of *Gracilaria pseudoverrucosa* agar (Gracilariaceae, Rhodophyta). *Carbohydr. Polym.* 8: 285–301.
- Lahaye, M., W. Yaphe, M. T. Phan Viet and C. Rochas. 1989. ^{13}C nuclear magnetic resonance investigation of methylated and charged agarose oligosaccharides and polysaccharides. *Carbohydr. Res.* in press.
- McLachlan, J. 1985. Macroalgae (seaweeds): industrial resources and their utilization. *Plant and Soil* 89: 137–157.

- Morrice, L. M., M. W. McLean, W. F. Long and F. B. Williams. 1983. Porphyrin primary structure. An investigation using β -agarase I from *Pseudomonas atlantica* and ^{13}C -NMR spectroscopy. *Eur. J. Biochem.* 133: 673–684.
- Nicolaisen, F. M., I. Meyland and K. Schaumburg. 1980. ^{13}C -NMR spectra at 67.9 MHz of agarose solutions and partly 6-O-methylated agarose at 95 °C. *Acta. Chem. Scand. Ser. B* 34: 103–107.
- Penman, A. and D. A. Rees. 1973. Carrageenans. Part IX. Methylation analysis of galactan sulphates from *Furcellaria fastigiata*, *Gigartina canaliculata*, *Gigartina chamissoi*, *Gigartina atropurpurea*, *Ahnfeltia durvillaei*, *Gymnogongrus furcellatus*, *Euclima isoforme*, *Euclima uncinatum*, *Aghardhiella tenera*, *Pachymenia hymantophora*, and *Gloiopeltis cervicornis*. Structure of ϵ -carrageenan. *J. Chem. Soc. (Perkin 1)*: 2182–2187.
- Rees, D. A. 1961. Enzymatic synthesis of the 3,6-anhydro-L-galactose within porphyran from L-galactose-6-sulphate units. *Biochem. J.* 81: 347–352.
- Rees, D. A. 1969. Structure, conformation and mechanism in the formation of polysaccharide gels and networks. *Advances Carbohydr. Chem. Biochem.* 24: 267–332.
- Rochas, C. and M. Lahaye. 1989 a. Average molecular weight and molecular weight distribution of agarose and agarose type polysaccharides. *Carbohydr. Polymers*. In press.
- Rochas, C. and M. Lahaye. 1989 b. Solid state ^{13}C -NMR spectroscopy of red seaweeds, agars and carrageenans. *Carbohydr. Polymers*. In press.
- Rochas, C., M. Lahaye, W. Yaphe and M. T. Phan Viet. 1986. ^{13}C -NMR-spectroscopic investigation of agarose oligomers. *Carbohydr. Res.* 148: 199–207.
- Usov, A. I., E. G. Ivanova and A. S. Shashkov. 1983. Polysaccharides of algae. XXXIII. Isolation and ^{13}C -NMR spectral study of some new gel-forming polysaccharides from Japan Sea red seaweeds. *Bot. Mar.* 26: 285–294.
- Usov, A. I., S. V. Yarotsky and A. S. Shashkov. 1980. ^{13}C -NMR spectroscopy of red algal galactans. *Biopolymers* 19: 977–990.