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THE CHEMISTRY AND IMMUNOCHEMISTRY OF CARRAGEENANS FROM *Eucheuma* AND RELATED ALGAL SPECIES*

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ABSTRACT

Carrageenans from several species of *Eucheuma* have been fractionated into KCl-soluble and KCl-insoluble fractions and analyzed by the usual chemical procedures. An anti-κ-carrageenan, the reactivity of which is directed to κ-structures (*i.e.*, 3-linked galactose 4-sulphate, and 4-linked 3,6-anhydrogalactose) was used to analyze these carrageenans immunochemically. The antibody preparation shows only a small amount of cross-reactivity with ι-type carrageenans and thus could be used to distinguish κ- and ι-type carrageenans, the latter having an index of homology of less than 0.2. A comparison of chemical and immunochemical data yielded further information as to the nature of the carrageenan–anti-carrageenan interaction, as well as elucidating the finer structure of carrageenans.

INTRODUCTION

The demonstration that carrageenans are immunogenic opened a new approach to their qualitative identification[1,2], which has been extended to allow quantitative characterization by the development of an agarose-medium turbidimetric assay for cross-reacting antigens[3]. The use of this technique in conjunction with the more common infrared and chemical procedures has proved to be useful in elucidating the finer structure of certain λ-type carrageenans[4,5]. In the present study, carrageenans were extracted from karyologically identified *Eucheuma* species and fractionated into KCl-soluble and KCl-insoluble carrageenans. Chemical and immunochemical procedures were then applied to characterize these polymers.

RESULTS AND DISCUSSION

Carrageenans of the kappa (κ) and iota (ι) types are composed ideally of alternating residues of D-galactose 4-sulphate (Gal-4-SO_4) and 3,6-anhydro-D-galactose (3,6-anGal). ι-Carrageenan is distinguished from κ-carrageenan by the

*Dedicated to Dr. Elizabeth Percival.

TABLE I

CHEMICAL ANALYSIS OF CARRAGEENANS

Sample	*Algal species and nuclear stage*	*Solubility of polysaccharide fraction in 0.3M KCl*	*Total recovered carrageenan* (%)	SO_4 (%)	*3,6-AnGal* (%)	*Increase* (%) *in 3,6-anGal*		*Molar ratios of Gal:3,6-anGal:SO_4*
						HO^-, BH_4^-	IO_4^-, HO^-, BH_4^-	
1	*E. cottonii* ♀	insol.	86.0	20.4	33.6	0	0	1:0.73:0.82
2		sol.	14.0	10.0	8.2	3.7	—	1:0.10:0.23
3	*E. cottonii* ⊕	insol.	81.4	19.0	29.5	0	0	1:0.59:0.70
4		sol.	18.6	11.5	6.0	3.3	—	1:0.07:0.27
5	*E. striatum* ♀[a]	insol.	76.4	20.0	24.2	0	0	1:0.45:0.67
6		sol.	23.6	17.0	9.2	0	0	1:0.13:0.43
7	*C. crispus* ♀	insol.	—	23.1	28.4	0	0	1:0.59:0.89
8	*E. serra* ♀	insol.	62.2	26.5	17.7	21.4	12.2	1:0.32:0.89
9		sol.	37.8	26.6	16.4	23.0	26.3	1:0.29:0.87
10	*E. serra* ⊕	insol.	66.1	31.0	18.5	13.0	15.1	1:0.38:1.2
11		sol.	33.9	25.0	15.9	14.2	18.4	1:0.27:0.79
12	*E. nudum* ♀	insol.	69.0	25.5	20.9	6.8	11.7	1:0.40:0.89
13		sol.	31.0	21.2		—	—	
14	*E. nudum* ⊕	insol.	67.6	26.7	20.4	10.5	13.6	1:0.39:0.95
15		sol.	32.4	23.9	17.4	17.8	19.7	1:0.30:0.76
16	*E. spinosum*	Marine colloids	—	→ As in Lawson *et al.*[7] ←				
17	*E. isiforme*	Marine colloids	—					
18	*A. tenera*	Marine colloids	—					

[a] *E. striatum* var Tamb.

occurrence of a sulphate group at position 2 of the 3,6-anhydride residue. This pattern may, however, be interrupted or masked by the occurrence of Gal-6-SO_4 or Gal-2,6-diSO_4, or even non-sulphated residues, which Rees has termed "kinking residues" because of their effect on polysaccharide conformation[6]. The presence of such residues causes the molar ratios of Gal:3,6-anGal:SO_4 to deviate from the expected 1:1:1 for κ-carrageenan and 1:1:2 for ι-carrageenan. The KCl-insoluble carrageenans contain enough κ- or ι-characteristics (*i.e.*, 3,6-anGal or 3,6-anGal-2-SO_4) to render them insoluble in aqueous KCl. If kinking residues reach a critical proportion relative to the 3,6-anhydride units, the polysaccharides become soluble in KCl[7]. We have thus fractionated the carrageenans from tetrasporic and carposporic algal plants of *E. cottonii*, *E. serra*, and *E. nudum*, and from carposporic plants of *E. striatum*, into KCl-soluble and KCl-insoluble carrageenans. The proportions of soluble and insoluble polysaccharides recovered and their chemical analyses are indicated in Table I.

Iota-type carrageenans

Infrared spectroscopy is used regularly to characterize the types of carrageenans obtained from different carrageenophytes. Figs. 1A and B show a portion of the infrared spectra of the various carrageenans used in this study. All the samples exhibit the characteristic band at 1240–1250 cm^{-1} for carbohydrate ester-sulphate. The spectra of KCl-soluble and KCl-insoluble carrageenans from *E. serra* and *E. nudum* extracted in this laboratory, as well as the spectra of carrageenans from *E. spinosum*, *E. isiforme*, and *Agardhiella tenera*, all contain the characteristic ι-carrageenan bands (Fig. 1B). The KCl-soluble and KCl-insoluble carrageenans from *E. serra* and *E. nudum* (samples 8–15, Table I) have similar molar ratios of Gal:3,6-anGal:SO_4. They contain 1.4–1.6 sulphate residues per disaccharide and thus conform closely to ι-type carrageenans. The KCl-soluble carrageenans from *E. serra* and *E. nudum* (samples 9, 11, 13, and 15, Table I) vary slightly from their respective KCl-insoluble counterparts (samples 8, 10, 12, 14, Table I). The former contain less 3,6-anGal and more kinking residues (Table II). Whether or not these slight differences can account for the different solubility properties in aqueous KCl is not clear. The majority of the kinking residues of the ι-type carrageenans are of the non-6-sulphated type. Most of the ι-carrageenans (samples 9–15, Table II) contain only Gal-2,6-diSO_4 in addition to the non-6-sulphated residues, whereas samples 8 and 16 contain both Gal-6-SO_4 and Gal-2,6-diSO_4 kinking residues.

Kappa-type carrageenans

The infrared spectra of the KCl-insoluble carrageenans from tetrasporic and carposporic plants of *E. cottonii* and from carposporic plants of *E. striatum* and *C. crispus* show the characteristic κ-type carrageenan bands (Fig. 1A). These carrageenans contain 0.8–0.9 sulphate residue per disaccharide, which conforms to what

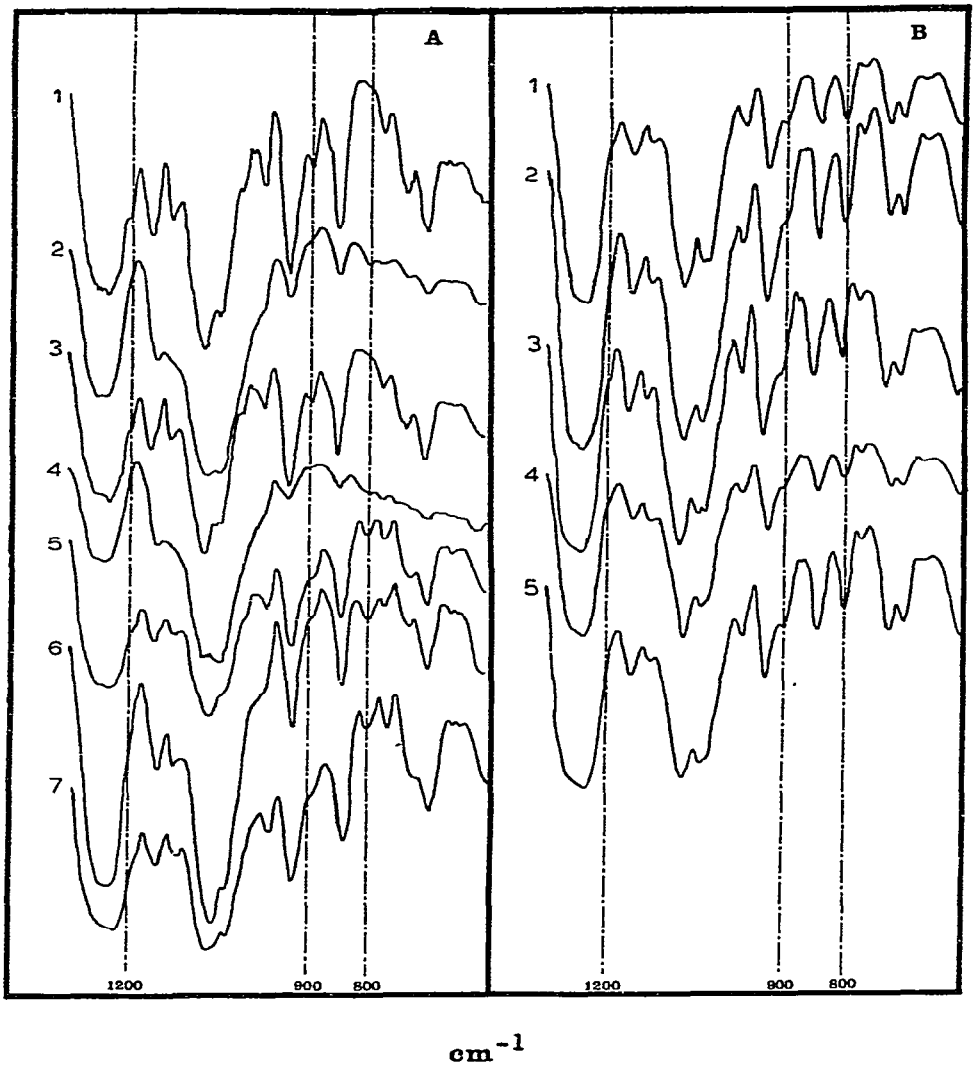

Fig. 1. A. The infrared spectra of κ-type carrageenans: 1, *E. cottonii* ♀ KCl-insoluble; 2, *E. cottonii* ♀ KCl-soluble; 3, *E. cottonii* ⊕ KCl-insoluble; 4, *E. cottonii* ⊕ KCl-soluble; 5, *E. striatum* ♀ KCl-insoluble; 6, *E. striatum* ♀ KCl-soluble; 7, *C. crispus* ♀ KCl-insoluble. B. The infrared spectra of ι-type carrageenans: 1, *E. spinosum*; 2, *E. isiforme*; 3, *E. serra*; 4, *E. nudum*; 5, *A. tenera*.

is expected for κ-type carrageenan. Of these carrageenans, however, only the KCl-insoluble carrageenan from carposporic *E. cottonii* approaches the theoretical molar ratios of 1:1:1 for Gal:3,6-anGal:SO_4. The infrared spectrum of the KCl-soluble carrageenan from *E. striatum* is qualitatively similar to that of the KCl-insoluble carrageenan extracted from the same plants. In general, the spectra for the KCl-soluble carrageenans from carposporic and tetrasporic plants of *E. cottonii* are

TABLE II

CHARACTERISTICS OF 4-LINKED "KINKING" RESIDUES

Sample	*Algal species and nuclear stage*	*Solubility of polysaccharide fraction in 0.3M KCl*	*Gal-6-SO_4* (%)	*Gal-2,6-diSO_4* (%)	*Non-6-SO_4* (%)
1	*E. cottonii* ♀	insol.	0	0	5.4
2		sol.	0.40	—	31.0
3	*E. cottonii* ⊕	insol.	0	0	9.6
4		sol.	0.30	—	33.5
5	*E. striatum* ♀	insol.	0	0	14.9
6		sol.	0	0	30.2
7	*C. crispus* ♀	insol.	0	0	10.7
8	*E. serra* ♀	insol.	2.5	4.5	10.5
9		sol.	0	7.9	12.2
10	*E. serra* ⊕	insol.	0	5.0	11.5
11		sol.	0	4.8	14.3
12	*E. nudum* ♀	insol.	0	3.0	—
13		sol.	—	—	—
14	*E. nudum* ⊕	insol.	0	4.5	9.9
15		sol.	0	6.5	11.9
16	*E. spinosum*	Marine colloids	2.1	4.3	11.9
17	*E. isiforme*	Marine colloids	0	5.9	11.3
18	*A. tenera*	Marine colloids	0	3.2	15.1

characteristic of κ-type carrageenan, in that they contain bands at 936 and 840 cm^{-1}, but differences from the spectra for κ- or ι-carrageenan are also evident (Fig. 1A). The KCl-soluble carrageenans from *Eucheuma* species that produce κ-type carrageenans differ chemically from their KCl-insoluble counterparts. As expected from their solubility properties, they contain less 3,6-anGal and less sulphate (Table I). They contain a large proportion of kinking residues, almost exclusively non-6-sulphated. The KCl-insoluble κ-type carrageenans from these species also contain the non-6-sulphated type of kinking residues.

Immunochemistry

It has been suggested[2] that the reactivity of an antiserum to κ-carrageenan was directed to some structural feature associated with 3,6-anGal. The antibody preparation derived from the same antiserum, and prepared as described, demonstrates not only that it is directed to a structural feature associated with 3,6-anGal but also that it reacts only when 3,6-anGal is found in association with Gal-4-SO_4. If either of these residues is absent from the polymer, the antibody will not react[8]. We have also found[8] that 2-sulphation of the 3,6-anhydride reduces the reactivity, in spite of the

presence of Gal-4-SO_4. It is possible, therefore, to use this antibody preparation to distinguish κ- and ι-carrageenans, and to probe the chemical and structural relations between κ-type carrageenans.

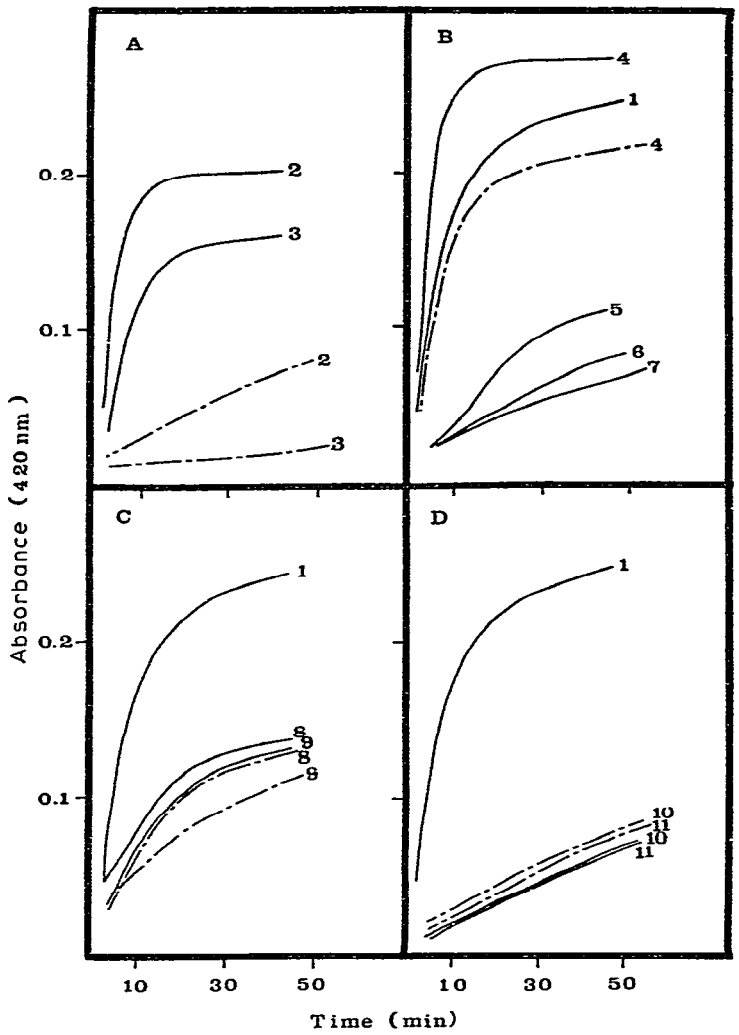

Fig. 2. The reactions of KCl-soluble (-·---) and KCl-insoluble (———) carrageenans from 1, *C. crispus* ♀; 2, *E. cottonii* ♀; 3, *E cottonii* λ; 4, *E. striatum* ♀; 5, *E. isiforme*; 6, *E. spinosum*; 7, *A. tenera*; 8, *E. nudum* ♀; 9, *E. nudum* ⊕; 10, *E. serra* ♀; and 11, *E. serra* ⊕.

Equivalent amounts of the various carrageenans were treated with equal amounts of antibody. The interaction of the antigen and antibody was quantified by the agarose-medium turbidimetric assay, and the results are shown in Fig. 2. The ability of the antibody preparation to discriminate between κ- and ι-carrageenans is

clearly indicated. KCl-insoluble carrageenans from tetrasporic and carposporic plants of *E. cottonii* (Fig. 2A) and from carposporic plants of *E. striatum* and *C. crispus* (Fig. 2B) react much better than do the *ι*-type carrageenans (Figs. 2B, C, and

TABLE III

IMMUNOCHEMICAL HOMOLOGY OF THE VARIOUS CARRAGEENANS TO *C. crispus* κ-TYPE CARRAGEENAN

Sample	*Algal species and nuclear stage*	*Solubility of polysaccharide fraction in 0.3M KCl*	*Rate (Absorbance/min) × 10^3 (±10%)*	*Total turbidity (±10%)*	*I.H.*
1	*E. cottonii* ♀	insol.	29	0.20	1.3
2		sol.	1.5	0.12	0.04
3	*E. cottonii* ⊕	insol.	15	0.16	0.53
4		sol.	0	0	0
5	*E. striatum* ♀	insol.	42.5	0.275	2.6
6		sol.	10.0	0.225	0.9
7	*C. crispus* ♀	insol.	18	0.25	1
8	*E. serra* ♀	insol.	1.3	0.10	0.03
9		sol.	1.3	0.10	0.03
10	*E. serra* ⊕	insol.	1.3	0.10	0.03
11		sol.	1.3	0.10	0.03
12	*E. nudum* ♀	insol.	5.5	0.14	0.16
13		sol.	2.5	0.13	0.07
14	*E. nudum* ⊕	insol.	4.8	0.13	0.14
15		sol.	5.5	0.13	0.16
16	*E. spinosum*	Marine colloids	1.6	0.10	0.04
17	*E. isiforme*	Marine colloids	4.0	0.125	0.10
18	*A. tenera*	Marine colloids	1.0	0.125	0.03

D). Table III contains the immunochemical data, and the indices of homology (I.H.) of the various carrageenans calculated by the following formula:

$$\text{I.H.} = \frac{\text{experimental (precipitation rate} \times \text{equilibrium absorbance)}}{\text{reference (precipitation rate} \times \text{equilibrium absorbance)}},$$

where the reference sample is the homologous antigen (*i.e.*, *C. crispus* carposporic KCl-insoluble carrageenan). The soluble carrageenans from *E. cottonii* are not only chemically but also immunochemically different from the KCl-insoluble carrageenan. The difference between the I.H. of these carageenans and the homologous antigen cannot be explained only by their lower contents of 3,6-anGal and SO_4, since the KCl-soluble carrageenan from *E. striatum*, although differing chemically from the KCl-insoluble carrageenans of *E. striatum* and *C. crispus*, has an I.H. value of 0.9 to the homologous antigen. This apparent discrepancy can be easily explained when one considers that only a small proportion of the 3,6-anGal residues of the

homologous antigen are actually involved in antibody binding[2]. It is suggested that the KCl-soluble carrageenan from *E. striatum* differs from the KCl-soluble carrageenan from *E. cottonii*, in that the 3,6-anGal and Gal-4-SO_4 residues in the former carrageenan are consecutively arranged in segments sufficiently long to constitute κ-type determinants; in the latter, these residues may be dispersed, and never occur consecutively, and the polymers therefore contain few or no κ-type determinants. This situation is also indicated by the fact that the *E. striatum* carrageenan contains 0.8 sulphate residue per disaccharide, as opposed to 0.42–0.50 sulphate residue per disaccharide in the KCl-soluble carrageenans from *E. cottonii*. The KCl-soluble carrageenan from *E. striatum* is, however, only half as reactive as its KCl-insoluble counterpart. Therefore, the carrageenans from *E. striatum* are more efficient at binding antibody than is the homologous antigen. This suggests that the κ-type carrageenans from *E. cottonii* and *C. crispus* also contain cryptic antibody binding-sites (*i.e.*, potential antigenic determinants not available for antibody binding, because of the molecular configuration of the particular polymer). In other words, both the content and availability of antigenic determinants are important in the interaction between antigen and antibody.

Conclusions

1. The KCl-soluble carrageenan from *E. cottonii*, which produces κ-type carrageenan, differs chemically and immunochemically from its KCl-insoluble counterpart. The KCl-soluble carrageenan from *E. striatum* contains a sufficient proportion of kinking residues to render it soluble in KCl, but it also contains sufficient κ-type segments to allow significant reactivity with the anti-κ-carrageenan. We postulate that the differences in immunological reactivity between *E. cottonii* and *E. striatum* KCl-soluble carrageenans are due to the internal organization of the κ-type residues.

2. The ability of some κ-carrageenans to react differently from other κ-type carrageenans suggests the existence of cryptic antibody binding-sites in the less reactive carrageenans. Binding sites may be rendered cryptic by the molecular configuration of the particular polysaccharide, as determined by the internal organization of kinking residues and κ-type residues. Another possibility is the extent of sulphation of the 3,6-anhydride.

3. The anti-κ-carrageenan preparation can be used to distinguish κ- from ι-carrageenans. Iota carrageenans show indices of homology of less than 0.2.

EXPERIMENTAL

Chemical treatments. — Alkaline borohydride modification and periodate oxidation were carried out as described by Lawson *et al.*[7].

Analyses. — Sulphate was determined by the method of Jones and Letham[9], and 3,6-anhydrogalactose by the resorcinol method of Yaphe and Arsenault[10]. As

carrageenans consist of the three components, galactose, 3,6-anhydrogalactose, and sulphate, galactose was estimated by difference.

Gal-6-SO_4 and Gal-2,6-diSO_4 were determined, as suggested by Lawson *et al.*[7], by determination of the increase in 3,6-anGal residues on treatment with hot, alkaline borohydride, before and after exposure to periodate. The increase in 3,6-anGal arises from elimination of an equimolar amount of Gal-6-SO_4. Periodate-oxidizable residues are Gal-6-SO_4, whereas periodate-resistant residues are Gal-2,6-diSO_4.

Infrared spectroscopy. — I.r.-spectral analysis was performed on carrageenan films formed by dissolving 3 mg of carrageenan with boiling water on AgCl discs, as described by Craigie and Leigh[11].

Antibody preparation. — γG-globulin anti-κ-carrageenan was prepared as previously described for a γG-globulin anti-λ-carrageenan[4].

Antigen–antibody reactions. — Quantitative antigen–antibody reactions were performed by the agarose-medium turbidimetric assay[3]; 250 μl of Ab was treated with 25 μg of Ag at 37° in a medium consisting of 0.1% of agarose in 0.01M PBS (pH 7.5, 3 ml). The rate of precipitation was recorded by an external-recorder readout attached to a Unicam 1800 spectrophotometer set at 420 nm.

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REFERENCES

1 K. H. JOHNSTON AND E. L. MCCANDLESS, *J. Immunol.*, 101 (1968) 556–562.
2 S. P. C. HOSFORD AND E. L. MCCANDLESS, *Can. J. Bot.*, 23 (1975) 2834–2841.
3 V. DININNO AND E. L. MCCANDLESS, *J. Immunol. Methods*, 17 (1977) 73–79.
4 V. DININNO AND E. L. MCCANDLESS, *Carbohydr. Res.*, (1978), in press.
5 E. L. MCCANDLESS, M. J. EVELEGH, C. M. VOLLMER, AND V. DININNO, *Proc. Int. Seaweed Symp.*, 9 (1978), in press.
6 D. A. REES, I. W. STEELE, AND F. B. WILLIAMSON, *J. Polym. Sci., Part C, Polym. Symp.*, (1969) 261–276.
7 C. J. LAWSON, D. A. REES, D. J. STANCIOFF, AND N. F. STANLEY, *J. Chem. Soc., Perkin Trans. 1*, (1973) 2177–2182.
8 V. DININNO AND E. L. MCCANDLESS, *Immunochemistry*, 15 (1978), in press.
9 A. S. JONES AND D. S. LETHAM, *Chem. Ind. (London)*, (1954) 662–663.
10 W. YAPHE AND G. P. ARSENAULT, *Anal. Biochem.*, 13 (1965) 143–148.
11 J. S. CRAIGIE AND C. LEIGH, *Carrageenans and Agar*, in J. A. HELLEBUST AND J. S. CRAIGIE (Eds.), *Handbook of Phycological Methods*, Vol. 2, Cambridge University Press, Cambridge, 1978, 109–131.