Carbohydrate Polymers **18** (1992) 237–242

The ^{13}C NMR spectroscopy of carrageenans: calculation of chemical shifts and computer-aided structural determination

Carlos A. Stortz* & Alberto S. Cerezo*‡

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

(Received 30 May 1991; revised version received 20 August 1991; accepted 3 September 1991)

The set of ^{13}C NMR absorptions produced by all the carbons of the diads potentially present in carrageenans is reported. They were obtained by calculation for unreported diads plus the compilation of up-to-date chemical shift data. A computer program was developed in order to aid in the matching of experimental data to the chemical shift data bank reported here.

INTRODUCTION

The first systematic studies of the ^{13}C NMR of carrageenans started around 1977 by the groups of Usov and Yaphe (Yarotskii *et al.*, 1977, 1978; Bhattacharjee *et al.*, 1978, 1979). By 1980, Usov and coworkers had fully assigned the spectra of κ-, ι-, Ω-, desulfated λ-, and desulfated κ-carrageenans (Usov *et al.*, 1980).

Further work of Bellion *et al.* (1981, 1983), Rochas *et al.* (1980, 1983), Usov (1984), Yaphe and coworkers (Greer & Yaphe, 1984; Greer *et al.*, 1985) and Mollion *et al.* (1986, 1988), among others, resulted in reports of new spectra of characterized carrageenans and/or oligosaccharides derived from them. Their approach was focused on the characterization of 'pure' samples resulting in spectra with 12 peaks. Methylation studies have shown (Stortz & Cerezo, 1986, 1988) that carrageenans also contain additional minor structural features, which may influence important properties. In order to identify these minor components, it is necessary to have the whole set of possible absorptions produced by all the diads potentially present. This was obtained by calculation for the units not yet determined and an up-to-date compilation of the ^{13}C NMR chemical shift data produced. A computer program was developed as an aid to matching experimental data with the chemical shift database.

*Research Member of the National Research Council of Argentina (CONICET).
‡To whom correspondence should be addressed.

Carbohydrate Polymers 0144-8617/92/$05.00

RESULTS AND DISCUSSION

Table 1 shows the monomeric structural units that are found in carrageenans. Table 2 shows the diads that can be formed from these structural units, assuming an alternating $\alpha(1 \rightarrow 3)$, $\beta(1 \rightarrow 4)$ sequence.

Building of the database

Table 3 shows the chemical shift data calculated or reported for the 42 diads shown in Table 2. The database should have been based on the triads potentially present, as the chemical shifts of a unit in a polysaccharide should depend both on the preceding and the following units. Nevertheless, the data available suggest that the effect of the former must be very small

Table 1. Usual structural units found in carrageenans

3-Linked β-D-Gal
3-Linked β-D-Gal 2-sulfate
3-Linked β-D-Gal 4-sulfate
3-Linked β-D-Gal 6-sulfate
3-Linked β-D-Gal 2,4-disulfate
3-Linked β-D-Gal 2,6-disulfate
3-Linked β-D-Gal 4,6-disulfate
4-Linked 3,6-An-α-D-Gal
4-Linked 3,6-An-α-D-Gal 2-sulfate
4-Linked α-D-Gal
4-Linked α-D-Gal 2-sulfate
4-Linked α-D-Gal 6-sulfate
4-Linked α-D-Gal 2,6-disulfate

Table 2. Diads that can be formed from the structural units found in carrageenans

	Unit A 3-linked (β)	Unit B 4-linked (α)	Name
I	Galactose	3,6-AnGal	β-
II	Galactose	3,6-AnGal 2-sulfate	α-
III	Galactose	Galactose	Desulf. λ-
IV	Galactose	Galactose 2-sulfate	
V	Galactose	Galactose 6-sulfate	γ-
VI	Galactose	Galactose 2,6-disulfate	δ-
VII	Galactose 2-sulfate	3,6-AnGal	
VIII	Galactose 2-sulfate	3,6-AnGal 2-sulfate	Alk. tr. λ-
IX	Galactose 2-sulfate	Galactose	
X	Galactose 2-sulfate	Galactose 2-sulfate	ξ-
XI	Galactose 2-sulfate	Galactose 6-sulfate	
XII	Galactose 2-sulfate	Galactose 2,6-disulfate	λ-
XIII	Galactose 4-sulfate	3,6-AnGal	κ-
XIV	Galactose 4-sulfate	3,6-AnGal 2-sulfate	ι-
XV	Galactose 4-sulfate	Galactose	
XVI	Galactose 4-sulfate	Galactose 2-sulfate	
XVII	Galactose 4-sulfate	Galactose 6-sulfate	μ-
XVIII	Galactose 4-sulfate	Galactose 2,6-disulfate	ν-
XIX	Galactose 6-sulfate	3,6-AnGal	Ω-
XX	Galactose 6-sulfate	3,6-AnGal 2-sulfate	
XXI	Galactose 6-sulfate	Galactose	
XXII	Galactose 6-sulfate	Galactose 2-sulfate	
XXIII	Galactose 6-sulfate	Galactose 6-sulfate	
XXIV	Galactose 6-sulfate	Galactose 2,6-disulfate	
XXV	Galactose 2,4-disulfate	3,6-AnGal	
XXVI	Galactose 2,4-disulfate	3,6-AnGal 2-sulfate	
XXVII	Galactose 2,4-disulfate	Galactose	
XXVIII	Galactose 2,4-disulfate	Galactose 2-sulfate	
XXIX	Galactose 2,4-disulfate	Galactose 6-sulfate	
XXX	Galactose 2,4-disulfate	Galactose 2,6-disulfate	
XXXI	Galactose 2,6-disulfate	3,6-AnGal	
XXXII	Galactose 2,6-disulfate	3,6-AnGal 2-sulfate	
XXXIII	Galactose 2,6-disulfate	Galactose	
XXXIV	Galactose 2,6-disulfate	Galactose 2-sulfate	
XXXV	Galactose 2,6-disulfate	Galactose 6-sulfate	
XXXVI	Galactose 2,6-disulfate	Galactose 2,6-disulfate	
XXXVII	Galactose 4,6-disulfate	3,6-AnGal	
XXXVIII	Galactose 4,6-disulfate	3,6-AnGal 2-sulfate	
XXXIX	Galactose 4,6-disulfate	Galactose	
XL	Galactose 4,6-disulfate	Galactose 2-sulfate	
XLI	Galactose 4,6-disulfate	Galactose 6-sulfate	
XLII	Galactose 4,6-disulfate	Galactose 2,6-disulfate	

and not detectable (for the only exception, see the footnote to Table 3). The use of diads is therefore at the moment the more realistic approach to the problem.

In building Table 3, the data of Welti (pers. comm. to Usov, 1984) were considered for the assignments of κ- and ι-carrageenans, adding an offset of +0·8 ppm to allow for different chemical shift referencing with our apparatus. For β- (desulfated κ-) and Ω-carrageenans, the data of Usov and coworkers (Yarotskii *et al.*, 1978; Usov *et al.*, 1980) were used, reversing the assignments for A-2 and G-2, and for A-3 and A-4, as suggested later (Rochas *et al.*, 1983; Usov, 1984), and modifying slightly some δ values using the data of Mollion *et al.* (1986). For desulfated λ-carrageenan the data of Usov *et al.* (1980) were used; for the three last carrageenans, an offset of +0·5 ppm was added. Thus, five reported diads serve as a base for the calculations of the possible chemical shift data for the rest of the diads. This was performed by several different methods.

Method a uses the shifts known for three reported diads A–B′, A′–B and A′–B′ to calculate the δ for a diad A–B. It served for four more diads.

$$\delta_{AB} = \delta_{AB'} + \delta_{A'B} - \delta_{A'B'}$$

e.g.

$$\delta_{\beta Gal4S-\alpha Gal} = \delta_{\beta Gal-\alpha Gal} + (\delta_{\beta Gal4S-3,6AnGal} - \delta_{\beta Gal-3,6AnGal})$$

This was considered the most reliable of the calculation methods, as it only uses correlations of carrageenan data.

Table 3. Chemical shifts reported or calculated for the possible diads of carrageenans

Diad	Unit A						Unit B						Method	
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6	A	B
I	103·0	70·3	80·9	66·8	75·8	61·8	95·1	70·7	79·8	78·5	77·3	70·0	—	(1)[a] —
II	102·7	70·1	79·0	66·8	75·9	61·8	92·1	75·9	79·0	78·5	77·6	70·3	—	a —
III	105·3	71·1	79·5	66·2	75·9	61·7	96·7	69·7	71·4	79·0	71·1	62·0	—	(2)[a] —
IV	105·3	71·1	79·5	66·2	75·9	61·7	94·7	76·9	69·3	79·1	70·9	61·8	=A-III	b′
V	105·3	71·1	79·5	66·2	75·9	61·7	96·7	69·5	70·9	79·0	68·8	68·3	=A-III	b′
VI	105·3	71·1	79·5	66·2	75·9	61·7	94·7	76·7	68·8	79·1	68·6	68·1	=A-III	b′
VII	101·4	78·5	79·8	66·9	75·8	61·7	95·1	70·7	79·8	78·5	77·3	70·0	b	=B-I
VIII	101·1	78·3	77·9	66·9	75·9	61·8	92·1	75·9	79·0	78·5	77·6	70·3	d	=B-II
IX	103·7	79·3	78·4	66·3	75·9	61·6	96·7	69·7	71·4	79·0	71·1	62·0	b	=B-III
X	103·7	79·3	78·4	66·3	75·9	61·6	94·7	76·9	69·3	79·1	70·9	61·8	=A-IX	=B-IV
XI	103·7	79·3	78·4	66·3	75·9	61·6	96·7	69·5	70·9	79·0	68·8	68·3	=A-IX	=B-V
XII	103·7	79·3	78·4	66·3	75·9	61·6	94·7	76·7	68·8	79·1	68·6	68·1	=A-IX	=B-VI
XIII	103·0	70·1	79·3	74·5[b]	75·2	61·8	95·7	70·3	79·6	78·8	77·3	70·0	—	(3)[a] —
XIV	102·7	69·9	77·4	72·7	75·3	61·8	92·7	75·5	78·4	78·8	77·6	70·3	—	(3)[a] —
XV	105·3	70·8	78·8	73·9	75·3	61·7	98·2	69·3	71·2	79·3	71·1	62·0	—	a —
XVI	105·3	70·8	80·5	73·9	75·3	61·7	98·8	76·5	69·1	79·4	70·9	61·8	=A-XV	d′
XVII	105·3	70·8	78·8	73·9	75·3	61·7	98·2	69·1	70·7	79·3	68·8	68·3	=A-XV	d′
XVIII	105·3	70·8	80·5	73·9	75·3	61·7	98·8	76·3	68·6	79·4	68·6	68·1	=A-XV	d′
XIX	103·1	70·1	80·7	66·5	73·5	67·6	95·1	70·5	79·9	78·9	77·3	69·9	—	(1)[a] —
XX	102·8	69·9	78·8	66·5	73·4	67·6	92·1	75·7	78·7	78·9	77·6	70·2	—	a —
XXI	105·4	71·1	79·3	65·9	73·6	67·5	96·7	69·7	71·5	79·4	71·1	61·9	—	a —
XXII	105·4	71·1	79·3	65·9	73·6	67·5	94·7	76·9	69·4	79·5	70·9	61·7	=A-XXI	d′
XXIII	105·4	71·1	79·3	65·9	73·6	67·5	96·7	69·5	70·8	79·4	68·8	68·2	=A-XXI	d′
XXIV	105·4	71·1	79·3	65·9	73·6	67·5	94·7	76·7	68·7	79·5	68·6	68·0	=A-XXI	d′
XXV	101·4	78·3	78·2	74·6	75·2	61·7	95·7	70·3	79·6	78·8	77·3	70·0	c	=B-XIII
XXVI	101·1	78·1	76·3	72·8	75·3	61·7	92·7	75·5	78·4	78·8	77·6	70·3	c	=B-XIV
XXVII	103·7	79·0	77·7	74·0	75·3	61·6	98·2	69·3	71·2	79·3	71·1	62·0	d	=B-XV
XXVIII	103·7	79·0	79·4	74·0	75·3	61·6	98·8	76·5	69·1	79·4	70·9	61·8	=A-XXVII	=B-XVI
XXIX	103·7	79·0	77·7	74·0	75·3	61·6	98·2	69·1	70·7	79·3	68·8	68·3	=A-XXVII	=B-XVII
XXX	103·7	79·0	79·4	74·0	75·3	61·6	98·8	76·3	68·6	79·4	68·6	68·1	=A-XXVII	=B-XVIII
XXXI	101·5	78·3	79·6	66·6	73·5	67·5	95·1	70·5	79·9	78·9	77·3	69·9	c	=B-XIX
XXXII	101·2	78·1	77·7	66·6	73·4	67·5	92·1	75·7	78·7	78·9	77·6	70·2	d	=B-XX
XXXIII	103·8	79·3	78·2	66·0	73·6	67·4	96·7	69·7	71·5	79·4	71·1	61·9	d	=B-XXI
XXXIV	103·8	79·3	78·2	66·0	73·6	67·4	94·7	76·9	69·4	79·5	70·9	61·7	=A-XXXIII	=B-XXII
XXXV	103·8	79·3	78·2	66·0	73·6	67·4	96·7	69·5	70·8	79·4	68·8	68·2	=A-XXXIII	=B-XXIII
XXXVI	103·8	79·3	78·2	66·0	73·6	67·4	94·7	76·7	68·7	79·5	68·6	68·0	=A-XXXIII	=B-XXIV
XXXVII	103·1	69·9	79·1	74·2	72·9	67·6	95·7	70·1	79·7	79·2	77·3	69·9	—	a′ —
XXXVIII	102·7	69·8	77·2	72·5	72·9	68·1	92·7	75·5	78·4	78·8	77·6	70·3	c	=B-XIV
XXXIX	105·3	71·0	80·0	73·8	72·7	67·5	98·2	69·3	71·2	79·3	71·1	62·0	d	=B-XV
XL	105·3	71·0	81·7	73·8	72·7	67·5	98·8	76·5	69·1	79·4	70·9	61·8	=A-XXXIX	=B-XVI
XLI	105·3	71·0	80·0	73·8	72·7	67·5	98·2	69·1	70·7	79·3	68·8	68·3	=A-XXXIX	=B-XVII
XLII	105·3	71·0	81·7	73·8	72·7	67·5	98·8	76·3	68·6	79·4	68·6	68·1	=A-XXXIX	=B-XVIII

[a](1) Data from Usov *et al.* (1980) corrected (see text). (2) Data from Usov *et al.* (1980). (3) Data from Welti (pers. comm. to Usov, 1984).
[b]This signal shifts to 72·7 ppm if the 3,6-AnGal unit previous to unit A is sulfated on C-2 (Yarotskii *et al.*, 1978).

Methods b and b′ add the displacement produced by sulfation on the monosaccharide β-D-galactopyranose (Archbald *et al.*, 1981) (method b) and on methyl α-D-galactopyranoside (Ruiz Contreras *et al.*, 1988) (method b′) to a parent non-sulfated carrageenan.

b: $\delta_{AsB} = \delta_{AB} + (\delta_{\beta GalS} - \delta_{\beta Gal})$

b′: $\delta_{ABs} = \delta_{AB} + (\delta_{Me\alpha GalS} - \delta_{Me\alpha Gal})$

Method c applies for disulfated units; it adds the influence of sulfation on monosaccharides to a parent monosulfated unit of carrageenan; it assumes there is no extra effect for the presence of two sulfates together on the monosaccharide.

$$\delta_{As'B} = \delta_{AsB} + (\delta_{\beta GalS'} - \delta_{\beta Gal})$$

e.g.

$$\delta_{\beta Gal2,6diS-3,6AnGal} = \delta_{\beta Gal6S-3,6AnGal} + (\delta_{\beta Gal2S} - \delta_{\beta Gal})$$

Another method used for one disulfated unit (diad XXXVII) was called a′, as it only uses comparisons between carrageenans, and not sulfation influence on monosaccharides:

$$\delta_{Ass'B} = \delta_{AsB} + \delta_{As'B} - \delta_{AB}$$

e.g.

$$\delta_{\beta Gal4,6diS-3,6AnGal} = \delta_{\beta Gal4S-3,6AnGal} + \delta_{\beta Gal6S-3,6AnGal} - \delta_{\beta Gal-3,6AnGal}$$

Methods d and d′ are combinations of the method a plus b (or b′); they are equivalent to performing a method a calculation on a diad previously determined by methods b or b′ (or vice versa). Method d can be expressed as:

$$\delta_{Ass'B} = \delta_{AB} + (\delta_{AsB'} - \delta_{AB'}) + (\delta_{\beta GalS'} - \delta_{\beta Gal})$$

or

$$\delta_{AsB} = \delta_{AB'} + (\delta_{A'B} - \delta_{A'B'}) + (\delta_{\beta GalS} - \delta_{\beta Gal})$$

and method d′ as:

$$\delta_{ABs} = \delta_{A'B} + (\delta_{AB'} - \delta_{A'B'}) + (\delta_{Me\alpha GalS} - \delta_{Me\alpha Gal})$$

As methods d and d′ add two different displacements on a base carrageenan, they can be regarded as the least reliable. Roughly, it can be considered that the reliability of the methods is a > b′ > b > a′ > c > d′ > d. The primed d and b methods are considered somehow more reliable than the unprimed, because the effects are measured on glycosides and not on free sugars.

For some units, it is not possible, at this stage, to predict the influence of the neighboring unit. Thus, for all the β-galactoses linked to sulfated α-galactoses it was assumed that the chemical shift was the same as that of an equivalent β-galactose linked to a non-sulfated α-galactose. With the same criterion, for the units B linked to β-galactose 2-sulfate, 2,4-disulfate, 2,6-disulfate and 4,6-disulfate, it was assumed that the chemical shift was the same as for a unit B linked to a β-Gal, its 4-sulfate, its 6-sulfate, or again its 4-sulfate, respectively.

It is known that in ι-carrageenan, the signal for C-4 of unit A shifts −1·8 ppm when compared to κ-carrageenan, i.e. the sulfation of C-2 of unit B produces a notorious upfield shift of the neighboring C-4. In preparing Table 3, this displacement was not taken into account when C-4 of unit A was not sulfated (diads II, VIII, XX and XXXII), as it would shift the signal of C-4 to 64·8–65·1 ppm, where no carbon of a secondary alcohol is expected to appear.

There is another concern for the δ values of the C-1 of α-galactose 6-sulfate and 2,6-disulfate linked to β-galactose 4-sulfate (unit B, diads XVII and XVIII), as the data of Bellion *et al.* (1983) showed their appearance at 98·2 and 98·8 ppm (considering a constant offset of +0·7 ppm), i.e. 0·9 and 3·5 ppm downfield the place where they were supposed to appear, respectively. These resonances were confirmed (Furneaux & Miller, 1985), not only by the ^{13}C NMR data alone, but also corroborated by methylation analysis of a μ-/ν-carrageenan (Ciancia *et al.*, unpublished results). These displacements are also caused by the effect of the 4-sulfate on the β-galactose unit, probably changing the conformation by altering the preferred torsional angles around the glycosidic bond (conformational map), and thus modifying the spatial relationship between the H-1 of unit B and the H-3 of unit A, which may result in a considerable shift (Beierbeck *et al.*, 1977) (up to 4·5 ppm). The appearance of a peak around 80·5 ppm in the spectra of ν-carrageenans (Bellion *et al.*, 1983; Ciancia *et al.*, unpublished results) which disappears after alkaline treatment (Bellion *et al.*, 1983) confirms this suggestion by being assigned to C-3 of unit A. Probably for μ-carrageenans the displacement for C-3 is smaller, as it occurs with its corresponding C-1 (0·9 ppm).

The fact that the displacement is produced by the neighboring sulfate on C-4 is confirmed by: (a) the C-1 of an α-galactose 6-sulfate linked to β-galactose (unit B, diad V, γ-carrageenan) appears (Greer & Yaphe, 1984; Knutsen & Grasdalen, 1987) where it is expected to (96·7 ppm); (b) in the ^{13}C NMR of a λ-carrageenan (Bremond *et al.*, 1987) there are no peaks between 96 and 100 ppm, and the main one appears around 94 ppm, as expected (Table 3); (c) the fact that the sulfate on C-4 is axial, the same as the 2-sulfate on 3,6-anhydrogalactose, which is known to cause large effects on the neighboring unit (compare, for instance, unit A of diads XIII and XIV), and (d) the sulfation of C-4 of unit A of β-carrageenan produces a similar, but smaller displacement (0·6 ppm) on the C-1 of a neighboring 3,6-anhydrogalactose (cf. diads I and XIII).

In order to check the calculation methods, unit A of diads XIII and XIX were calculated from diad I using method b. Most of the experimental values fell within 0·3 ppm of the calculated ones, and only one signal in each unit deviated 0·5–0·6 ppm from that value.

Development of a computer program

Now this data is available, it is possible to use it to compare an experimental spectrum with each set. A computer program, CARRAG.EXE, written and compiled in Quick Basic (Microsoft) was developed in order to perform the comparison and match the data with the possible diads.

The program first reads the database (a sequential file equivalent to Table 3 named CARRAG.DAT) and then prompts the user to input the chemical shifts of the peaks of a given spectrum (up to 50), which may be introduced in any order. After sorting the peaks, the program does the following:

(a) For each peak, it searches for all the data that match within 0·7 ppm (or another user-defined range); then, it lists all the possible matches.
(b) For each of the possible 84 monomeric residues,

it looks to see if the six carbons appear within 0·7 ppm (or the other defined ε). If they do, for each unit j, it calculates a 'coincidence percentage', according to:

$$C_j = 100 \left(1 - \frac{\sum_{i=1}^{6} (\delta_{exp} - \delta_{ij})^2}{6(\varepsilon + 0{\cdot}01)^2} \right)$$

where i represents each of the six carbons and δ_{exp} is the chemical shift of the peak which is closer to the δ_{ij}. If any of the δ_{ij} goes beyond ε of an experimental peak, then C_j is not calculated. Applying this formula, a spectrum with the six peaks appearing exactly where the database assumes has a 100% coincidence; if the six peaks appear within 0·1 ppm the coincidence goes to 98·0% with ε = 0·7 ppm, and to 94·1% with ε = 0·4 ppm. On the other hand, if the six peaks are at the ε limit, the coincidence falls to less than 5%. A list of the units that match, and their respective 'coincidence percentages' is produced.

(c) Using the previously obtained data, the 'coincidence percentage' now for each diad is generated and listed. Only if the two units of the diad did effectively match, is a coincidence calculated as a geometric mean:

$$C_{jk} = (C_j C_k)^{0.5}$$

This third part is considered to be the most valuable part of the program. A printout can be obtained, either in full or just the third item. The output can also be directed to a text (ASCII) file.

Because of reports of new assignments or spectra it is important to be able to update the database. CARRAG.EXE provides for a way of modifying the file CARRAG.DAT or part of it. In addition a constant offset can be added or subtracted to the data, to allow for different chemical shift referencing resulting from the use of different equipment.

It should be pointed out that the program does not provide a way of introducing areas or intensities for each peak. However, as an option, the user is allowed to introduce two sets of data, the first one as 'major peaks' and the second one as 'minor peaks'. Besides the obvious semiquantitative significance of this classification, the major diads correspond (or define) 'ideal' or 'repetitive' structures, while the minor ones represent 'fine structures'. The program does not take into account the possible presence of end-chain reducing units arising from chemical degradation of the samples, or the small thermal degradation associated with the determination of spectra at high temperatures (Masson, 1955).

After sorting each set separately, the program:

(a) Matches both sets of peaks with the data.
(b) Calculates a 'coincidence percentage') with the 84 residues, initially only for the data entered as 'major peaks', and later for all the peaks.
(c) Calculates a 'coincidence percentage' first with the diads that match the major peaks, then with the major plus the minor peaks after subtracting the diads that are matched first.

In this way, the presence of diads in minor amounts within a main structure can be easily deduced.

METHODS

The computer program was written and compiled on an Olivetti M-24 computer using Quick Basic 4·0 (Microsoft). It should run on any IBM PC-compatible computer with any video card, disk drive or monitor. The printer has to support the IBM extended characters (code 128-255) to print Greek letters.

CONCLUSION

Carrageenans have an alternating $\alpha(1 \rightarrow 3)$, $\beta(1 \rightarrow 4)$ linear D-galactan structure; further differentiation of the structural units is produced by specific sulfation and by cyclization of the α-galactose residues into 3,6-anhydro derivatives. This scheme limits the structural possibilities to 42 diads; the reported ^{13}C NMR absorptions and the displacement produced by sulfation allow a complete set of chemical shifts for all these possible diads to be built up. This overall view of the ^{13}C NMR investigations of carrageenan structure has many advantages, namely:

(a) The comparison of experimental data with the previously determined set, aided by a computer program, allows the identification of major diads and minor diads (fine structure).
(b) The identification of new displacement effects when the experimental data do not match those calculated, as might have occurred with the reported absorptions of the C-1 of α-units in μ- and ν-carrageenans, may result in a better knowledge of the linkage conformation.
(c) There is the possibility of further development in this field as new experimental data and/or increases in resolution of the equipment will allow finer displacement effects to be recognized.

ACKNOWLEDGEMENTS

We are indebted to UMYMFOR (FCEyN-UBA) for technical assistance. This work was supported by grants from CONICET (PID 3040700/88), TWAS (BC-22) and UNESCO (871.716.9(2)).

REFERENCES

Archbald, P.J., Fenn, M.D. & Roy, A.B. (1981). *Carbohydr. Res.*, **93**, 177.

Beierbeck, H., Saunders, J.K. & ApSimon, J.W. (1977). *Can. J. Chem.*, **55**, 2813.

Bellion, C., Hamer, G.K. & Yaphe, W. (1981). *Proc. Int. Seaweed Symp.*, **10**, 379.

Bellion, C., Brigand, G., Prome, C., Welti, D. & Bociek, S. (1983). *Carbohydr. Res.*, **119**, 31.

Bhattacharjee, S.S., Yaphe, W. & Hamer, G.K. (1978). *Carbohydr. Res.*, **60**, C1.

Bhattacharjee, S.S., Yaphe, W. & Hamer, G.K. (1979). *Proc. Int. Seaweed Symp.*, **9**, 379.

Bremond, G. de L., Quillet, M. & Bremond, M. (1987). *Phytochemistry*, **26**, 1705.

Furneaux, R.H. & Miller, I.J. (1985). *Bot. Mar.*, **28**, 419.

Greer, C.W. & Yaphe, W. (1984). *Bot. Mar.*, **27**, 473.

Greer, C.W., Rochas, C. & Yaphe, W. (1985). *Bot. Mar.*, **28**, 9.

Knutsen, S.H. & Grasdalen, H. (1987). *Bot. Mar.*, **30**, 497.

Masson, C.R. (1955). *Can. J. Chem.*, **33**, 597.

Mollion, J., Moreau, S. & Christiaen, D. (1986). *Bot. Mar.*, **29**, 549.

Mollion, J., Morvan, H., Bellanger, F. & Moreau, S. (1988). *Phytochemistry*, **27**, 2023.

Rochas, C., Rinaudo, M. & Vincendon, M. (1980). *Biopolymers*, **19**, 2165.

Rochas, C., Rinaudo, M. & Vincendon, M. (1983). *Int. J. Biol. Macromol.*, **5**, 111.

Ruiz Contreras, R., Kamerling, J.P., Breg, J. & Vliegenthart, J.F.G. (1988). *Carbohydr. Res.*, **179**, 411.

Stortz, C.A. & Cerezo, A.S. (1986). *Carbohydr. Res.*, **145**, 219.

Stortz, C.A. & Cerezo, A.S. (1988). *Carbohydr. Res.*, **172**, 139.

Usov, A.I. (1984). *Bot. Mar.*, **27**, 189.

Usov, A.I., Yarotskii, S.V. & Shashkov, A.S. (1980). *Biopolymers*, **19**, 977.

Yarotskii, S.V., Shashkov, A.S. & Usov, A.I. (1977). *Bioorg. Khim.*, **3**, 1135.

Yarotskii, S.V., Shashkov, A.S. & Usov, A.I. (1978). *Bioorg. Khim.*, **4**, 745.