Carbohydrate Research, 163 (1987) 9–14
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

RAMAN SPECTROSCOPIC ANALYSIS OF THE SODIUM SALT OF KAPPA-CARRAGEENAN AND RELATED COMPOUNDS IN SOLUTION

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(Received October 28th, 1986; accepted for publication, December 22nd, 1986)

ABSTRACT

Laser-Raman spectra of Na^+ kappa-carrageenan, Na^+ neocarrabiose 4-sulphate, and neocarrabiose in the region 700–1500 cm^{-1} are reported for solutions in H_2O and D_2O. The C-1–H-1α vibration, coupled with C–OH related modes, is assigned to a band at 840 cm^{-1}, close to the maximum of the symmetrical C–O–S stretching (~850 cm^{-1}). The symmetrical S=O stretch is proposed to occur near 1040 cm^{-1} and is probably coupled with C–OH vibrations which give rise to strong bands in the region 1000–1100 cm^{-1}. The intense band in the region 730–740 cm^{-1} is ascribed to a complex ring vibration.

INTRODUCTION

Laser-Raman spectroscopy is an important tool for the structural analysis[1,2] of such biopolymers as polysaccharides and, unlike i.r. spectroscopy, it can be applied to aqueous solutions without interference by the solvent. However, the exact interpretation of vibration spectra is often difficult since all modes in the region below 1500 cm^{-1} are coupled[3–8]. Mathematically calculated predictions of the spectra are possible only for simple carbohydrates[4,6,7].

The structural analysis of galactans has been effected by ^{13}C-n.m.r. spectroscopy[9–11] and precise assignments could be achieved by using model compounds[10,12,13] prepared by enzymic hydrolysis[12,14,15]. We now report an analogous strategy for the interpretation of the Raman spectra of kappa-carrageenan and related compounds. I.r. data for kappa-carrageenan[13,16,17] and related compounds[13] have been reported.

EXPERIMENTAL

Solutions (50–90 mg/mL) of neocarrabiose (3) and sodium neocarrabiose 4-

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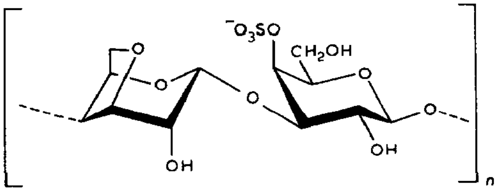
sulphate (**2**) (Sigma) in H_2O and D_2O were filtered (Millipore 0.22 μm) and then introduced into Kimax capillaries (i.d. 1 mm).

Kappa-carrageenan (Sigma, lot 1263) was purified by precipitation from an aqueous 1% solution with 2-propanol in the presence of NaCl. The precipitate was washed several times with 2-propanol–water (80:20), and the sodium form was obtained conventionally, using Amberlite IR-120 (Na^+), and isolated by freeze-drying.

Concentrations (typically 20 mg/mL) of the solutions of kappa-carrageenan in H_2O and D_2O are related to the percentage of galactose in the sample (determined by the phenol–sulphuric acid method[20]); concentrations >20 mg/mL gave solutions that were highly viscous at 27°.

Raman spectra were recorded with a Coderg double-monochromator spectrometer equipped with a Coherent Radiation argon ion laser, using the 514.5-nm excitation line. The scattered light was collected perpendicular to the incident beam and was subjected to photon counting. A 600-mW power supply was used with a spectral band-width of 5 cm^{-1} and a scan speed of 1 cm^{-1}/sec. After a 20-fold accumulation, each spectrum was smoothed and the data were stored in an Apple computer.



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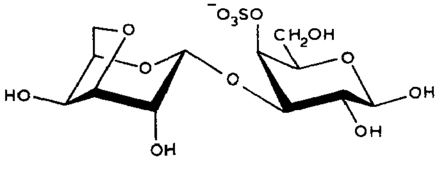

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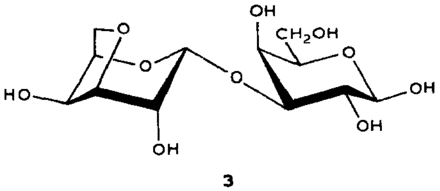

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RESULTS AND DISCUSSION

The repeating unit (neocarrabiose 4-sulphate) of kappa-carrageenan is →4)-3,6-anhydro-α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl 4-sulphate-(1→ (**1**).

The Raman spectra for solutions of Na$^+$ kappa-carrageenan (**1**), Na$^+$ neocarrabiose sulphate (**2**), and neocarrabiose (**3**) in H_2O and D_2O in the region 700–1500 cm^{-1} are given in Tables I and II. Peaks below 700 cm^{-1} are associated with skeleton and complex ring vibrations which differ for each carbohydrate[3,5,6] and are not considered.

Sulphate vibrational frequencies. — There are many i.r. data for polysaccharides in the solid state[16,18,21,22]. For kappa- and lambda-carrageenan, Bayley[16] assigned a peak in the region 820–840 cm^{-1} to the C–O–S vibration and suggested the strong absorption at 1240 cm^{-1} to be due to the S=O stretching vibration. The original view that the position of the C–O–S band depends only on the equatorial (820 cm^{-1}) or axial (840 cm^{-1}) orientation is not always true, since this band may also vary with the phase of the sample and the nature of other substituents on the pyranoid ring[23,24]. I.r. and Raman spectral data for glycosaminoglycans and model

TABLE I

OBSERVED FREQUENCIES (cm^{-1}) AND INTENSITIES[a] FOR THE RAMAN SPECTRA FOR SOLUTIONS IN H_2O IN THE REGION 700–1500 cm^{-1}

Na$^+$ Kappa-carrageenan	*Na$^+$ Neocarrabiose sulphate*	*Neocarrabiose*
		714 w
735 s	733 s	740 s
771 w	768 w	764 w
797 w		
	824 m (sh)	
843 s (sh)	836 vs	837 vs
850 vs	848 s (sh)	
		861 w
890 m	891 m	890 s
926 w	929 w	929 w
966 w	968 w	967 w
983 w		
	1002 w	991 w
1011 sh		
1038 vs	1039 vs	1042 m
1086 vs	1076 vs	1083 m
1120 m	1115 w (sh)	1119 w
1150 w	1148 br	1152 w
1200 w		1209 w
1250 br	1240 br	1274 br
1350 br	1340 br	
1460 br	1469 br	1468 br

[a]Key: vs, very strong; s, strong; m, moderate; w, weak; sh, shoulder; br, broad.

TABLE II

OBSERVED FREQUENCIES (cm^{-1}) AND INTENSITIES[a] FOR THE RAMAN SPECTRA FOR SOLUTIONS IN D_2O IN THE REGION 700–1500 cm^{-1}

Na⁺ Kappa-carrageenan	*Na⁺ Neocarrabiose sulphate*	*Neocarrabiose*
730 m	729 vs	736 s
769 w	768 m	763 m
794 w		
		817 sh
	829 s (sh)	825 s
845 s	847 s	
889 m	889 m	889 s
926 w	928 w	928 w
970 w	973 w	967 w
	1000 w	1000 w
1015 m	1015 m	
1048 s	1047 vs	1041 m
1058 sh		
1086 s	1088 s	1086 m
1119 m	1117 m	1119 m
1171 sh	1165 sh	1172 sh
1199 br	1196 br	1192 br
1257 br	1259 br	1267 m
		1288 m
		1322 w
1350 w	1345 w	1352 w
1425 br		
	1470 br	1468 br

[a] For key, see Table I.

compounds have been reported[25] and interpreted on the basis of a simplified model, according to which the Raman symmetrical C–O–S vibration should occur near 820 cm^{-1} and the antisymmetric vibration, strongly active in the i.r. near 980 cm^{-1}, should be weak. Our measurements on aqueous solutions of **1** and **2** also show no strong Raman band near 980 cm^{-1}. The region of the C–O–S Raman band[25] for kappa-carrageenan contains a maximum at 850 cm^{-1} and a shoulder at 843 cm^{-1}. Because of the non-linear base-line, the influence of deuteration upon the intensity of these peaks is difficult to verify.

More information on the position of the C–O–S band could be obtained from the spectra of solutions of **2** and **3** in H_2O and D_2O; in H_2O, **2** showed a maximum at 836 cm^{-1} and two shoulders at 824 and 848 cm^{-1}, and deuteration decreased the intensity of the maximum. Since **3** in H_2O has only one maximum at 837 cm^{-1}, and kappa-carrageenan does not have a band at 824 cm^{-1}, it is proposed that the Raman C–O–S symmetrical vibration for kappa-carrageenan in H_2O is at ~850 cm^{-1}. The origin of the peaks at 836 cm^{-1} for **2** and **3** and at 843 cm^{-1} for **1** is considered below, but the shoulder at 824 cm^{-1} for **2** cannot be explained at the present time.

The important Raman peak at 1060 cm^{-1} for dextran sulphate and heparin

(B-type)[25] due to S=O symmetrical vibrations is of low intensity in the i.r., but the antisymmetrical S=O vibrations give rise[16,21,25] to a strong i.r. band at 1230–1240 cm^{-1} which is nearly absent in the Raman spectra. Raman measurements on solutions of **1** and **2** in H_2O do not show a band at 1060 cm^{-1}, but two maxima appear at 1038 and 1086 cm^{-1} and 1039 and 1076 cm^{-1}, respectively; the second peaks are strongly influenced by deuteration and therefore have a contribution from C–OH modes. Moreover, **3** has a band at 1083 cm^{-1} which is less intense than that for kappa-carrageenan.

Comparison of the data for **2** and **3** shows that the presence of the sulphate group in **2** increases the intensity of the bands in the region 1000–1100 cm^{-1}. Since the band near 1038 cm^{-1} is intense for the sulphated compounds, but not for **3**, and since deuteration of **1** and **2** does not decrease the intensity of the band, it is proposed that a main contribution arises from the S=O symmetrical vibration. However, this first maximum is also partially due to a C–OH related mode since a less intense peak is observed at 1042 cm^{-1} for **3**. The situation for carrageenan is complicated by the fact that the bands in this region are dependent on the nature of the cation and can be influenced greatly by the nature and concentration of ions in solution, as shown by i.r. spectroscopy[19].

Vibration frequencies related to the anomeric carbon. — The bands for α anomers of monosaccharides and polysaccharides have been determined[3] as occurring at 840 ±7 cm^{-1}. Theoretical calculations[3,4,7] for α-D-glucopyranose show that the C-1–H-1 deformation is coupled with other C–H related modes and therefore is not influenced by O-deuteration. It is reasonable to assume that the sharp Raman peak at ~836 cm^{-1} for solutions of **2** and **3** in H_2O are partially due to the C-1–H-1 vibrations. However, this band is strongly influenced by deuteration which indicates, at least for **2** and **3**, the presence of a C–OH contribution.

The Raman spectrum of carrageenan contains a shoulder at 843 cm^{-1}, which is less intense relative to the C–O–S vibration than in the spectrum of **2**. Possibly, the difference in intensity reflects the involvement in glycosidic linkages of HO-1 and HO-4 of the β-D-galactopyranosyl 4-sulphate and the 3,6-anhydro-α-D-galactopyranosyl residues, respectively, and thus suggests also the coupling of the C-1–H-1α vibration with C–OH related modes for the disaccharides. However, the difference in intensity could also be due to the mutarotational equilibrium of the disaccharides.

The C-1–H-1β deformation band near 890 cm^{-1} has been proposed[7,26]. This band is observed for the spectra of **1**–**3** recorded for solutions in H_2O and D_2O. The difference in intensity of this band for the disaccharides cannot be due to the mutarotational equilibrium of the former, but indicates a coupling of the C-1–H-1β vibration with other modes.

Other bands. — The i.r. band at 930 cm^{-1} has been attributed to the presence of the 3,6-anhydro bridge[18], but our Raman measurements show only a very weak Raman band in this region. This aspect is being investigated further.

All the Raman spectra contained a strong band at 730–740 cm^{-1}, the intensity of which was little influenced by deuteration. This band is not given by other types of carbohydrate[3,8] and may be due to a characteristic, complex ring-vibration mode.

The region 1100–1500 cm^{-1} contains less intense and broad peaks. Bands in this region have been assigned for other carbohydrates[3-5]. Maxima near 1150 and 1350 cm^{-1} should correspond to CO stretchings and COH bending modes. A band near 1460 cm^{-1} has been attributed to a CH_2 bending vibration.

Little is known[3] about the appearance of O–D bands for carbohydrates in the Raman region 700–1500 cm^{-1}. On deuteration, O–H related modes are shifted towards lower frequencies. However, since almost all vibrations in this region are not pure, prediction of the position of new bands is difficult especially for such complex sugars as kappa-carrageenan and related compounds. The changes in the D_2O spectra of **3** (near 860 cm^{-1}) and the increase in intensity in the region 1400–1500 cm^{-1} for **2** remain to be explained. The broad maximum near 1200 cm^{-1} is due to Raman scattering of the solvent.

ACKNOWLEDGMENTS

Het Ministerie van Wetenschapsbeleid is thanked for a Prest-Mandaat (to T. M.), and Drs. J. P. Lafaut and J. Desmet for assistance with the laser-Raman equipment.

REFERENCES

1 F. S. Parker, *Applications of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry,* Plenum Press, New York, 1983.
2 A. T. Tu, *Raman Spectroscopy in Biology: Principles and Appliations,* Wiley, 1982.
3 P. D. Vasko, J. Blackwell, and J. L. Koenig, *Carbohydr. Res.*, 19 (1971) 297–310.
4 P. D. Vasko, J. Blackwell, and J. L. Koenig, *Carbohydr. Res.*, 23 (1972) 407–416.
5 H. Susi and J. S. Ard, *Carbohydr. Res.*, 37 (1974) 351–354.
6 M. Hineno, *Carbohydr. Res.*, 56 (1977) 219–227.
7 J. J. Cael, J. L. Koenig, and J. Blackwell, *Carbohydr. Res.*, 32 (1974) 79–91.
8 A. T. Tu, J. Lee, and F. P. Milanovich, *Carbohydr. Res.*, 76 (1979) 239–244.
9 A. I. Usov, S. V. Yarotsky, and A. S. Shashkov, *Biopolymers*, 19 (1980) 977–990.
10 C. W. Greer, C. Rochas, and W. Yaphe, *Bot. Mar.*, 28 (1985) 9–14.
11 C. Rochas, M. Rinaudo, and M. Vincendon, *Biopolymers,* 19 (1980) 2165–2175.
12 C. Rochas, M. Lahaye, and W. Yaphe, *Carbohydr. Res.*, 148 (1986) 199–207.
13 C. Rochas, M. Rinaudo, and M. Vincendon, *Int. J. Biol. Macromol.*, 5 (1983) 111–115.
14 C. W. Greer and W. Yaphe, *Proc. Int. Seaweed Symp., 11th*, (1984) 563–567.
15 C. Rochas and A. Heyraud, *Polym. Bull.*, 5 (1981) 81–86.
16 S. T. Bayley, *Biochim. Biophys. Acta,* 17 (1955) 194–205.
17 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.
18 D. J. Stancioff and N. F. Stanley, *Proc. Int. Seaweed Symp., 6th*, (1969) 595–609.
19 I. T. Norton, D. M. Goodwill, E. R. Morris, and D. A. Rees, *J. Chem. Soc., Faraday Trans. 1*, 79 (1983) 2475–2488.
20 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
21 S. F. D. Orr, *Biochim. Biophys. Acta,* 14 (1954) 173–181.
22 A. G. Lloyd, K. S. Dodgson, R. G. Price, and F. A. Rose, *Biochim. Biophys. Acta,* 46 (1962) 108–155.
23 M. J. Harris and J. R. Turvey, *Carbohydr. Res.*, 15 (1970) 51–56.
24 S. Peat, D. M. Bowker, and J. R. Turvey, *Carbohydr. Res.*, 7 (1968) 225–231.
25 F. Cabassi, B. Casu, and A. S. Perlin, *Carbohydr. Res.*, 63 (1978) 1–11.
26 A. T. Tu, J. Lee, and Y. C. Lee, *Carbohydr. Res.*, 67 (1978) 295–304.