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Acid and Enzymic Hydrolysis of Kappa Carrageenan

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SUMMARY

This paper describes the acid and enzymic hydrolysis of Kappa carrageenan and the gel permeation chromatography of the charged oligosaccharides.

Introduction

This paper concerns mainly the application of gel permeation chromatography to the investigation of hydrolysis of a purified kappa carrageenan and correlation with structural features. It was suggested by WEIGL and YAPHE (1966a) and WEIGL et al. (1966b) using a kappa and a iota carrageenases that every molecules of κ-carrageenan are not an homopolymer but a kappa-iota hybrid. In addition, they have isolated a κ-carrageenase which split specifically β (1 → 4) linked neocarrabiose units (Figure 1).

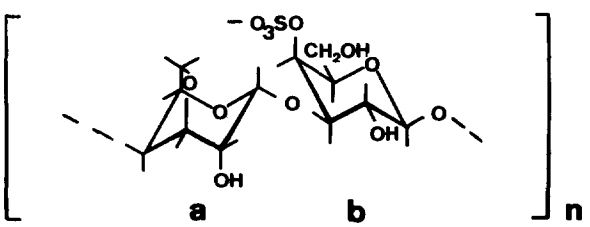

Fig. 1 : neocarrabiose sulfate unit

Soluble oligomers (n = 1,2,3,...) and an undegraded fraction are released after κ-carrageenase treatment. The lower oligomers have been previously separated by thin layer chromatography or,and gel permeation chromatography (WEIGL and YAPHE 1966a, Mc LEAN and WILLIAMSON 1979, BELLION 1980).

This paper deals with analytic and preparative chromatography of all products obtained by acid and enzymic hydrolysis.

Experimental

The κ-carrageenan sample is extracted from Euchema Cottonii and delivered by Sigma. κ-carrageenan is proposed to be a polymer $(ab)_n$; it has been purified as previously described (ROCHAS and RINAUDO 1980a, 1980b). Acid hydrolysis is performed on a 10g/l polymer solution at pH 3 (using H_2SO_4) by heating at 100° C under stirring. Aliquots are neutralized, filtered and chromatographed.

The κ-carrageenase was kindly given by YAPHE from Mc Gill University, Montreal, Canada. The enzymic hydrolysis was performed

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at pH 8.2 in 0,1 M NaCl, 0.005 M $NaHCO_3$ at 40° C. These conditions of pH and temperature seem to be optima following WEIGL and YAPHE (1966a), Mc LEAN and WILLIAMSON (1979). The hydrolysis was followed by oligomers liberation fractionated on Bio-Gel P6 and by viscosity decrease determined in a Ubbelohde viscosimeter.

Gel chromatography (G.P.C.) was used as the technique for the separation of oligomers. Acid hydrolysis studies have been performed on Bio-Gel P2 (column -400 mesh ; 210 x 1.5 cm) at 65°C. Enzymic hydrolysis has been studied with Bio-Gel P6 (column 200-400 mesh ; 150 x 2.5 cm) at 25° C. $NaNO_3$ solution was used as eluent and detection was performed with a differential refractive index monitor R 401 Waters. The parameters of the columns have been determined as previously described (HEYRAUD and RINAUDO 1978).

Results and Discussion

a) Acid hydrolysis. In Figure 2 schematic chromatograms for some times of hydrolysis are given. Over 150 hours there is no more higher molecular weight fraction (A) eluted at dead volume Vo.

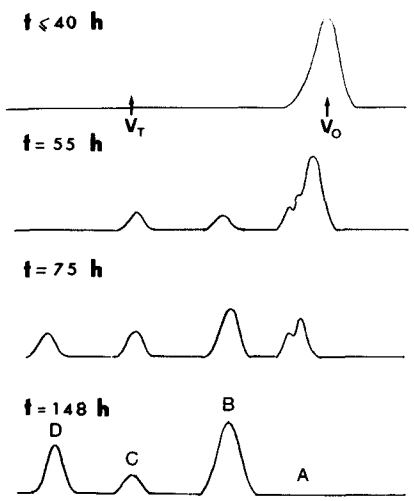

Fig. 2 : Chromatograms dependence with time of hydrolysis.

Total hydrolysis was obtained and the product were fractionated on preparative scale and identified. One recovers 64.9 (B), 14.1 (C) and 21 (D) weight per cent of each species.

The elution volume of fraction B located between the dead volume Vo and the total volume V_T is the same as that of toluen sulfonate sodium salt choosen as standard ; infrared spectrum shows a peak SO_3^- at 1250 cm^{-1} and a peak at 890 cm^{-1} attributed to $-O-SO_3^-$ axial by REES (1963), TURVEY and WILLIAMS (1962).

The result of the Dubois' method (DUBOIS et al. 1956) and the Nelson's colorimetric modification of Somogyi's method (WHISTLER and WOLFROM 1962) confirmed that it is a monosaccharide. The rotary power is $[\alpha]_D^{25}$ = + 57° and $[\alpha]_{300}^{25}$ = + 271° identic to D-galactose-4-sulfate synthesized by TURVEY and WILLIAMS (1962).

The ^{13}C NMR spectrum confirms that it is D-galactose-4-sulfate (ROCHAS et al. to be published).

The fraction C, eluted around V_T is D-galactose ; the structure is confirmed by IR and NMR spectroscopies.

The fraction D loosely adsorbed on the gel is eluted over V_T; it is 5-hydroxymethyl furfural as proved by ^{13}C, ^{1}H NMR and mass spectroscopies. This fraction corresponds to dehydration of monosaccharide (LEHMAN 1976). The results allow to conclude that under experimental conditions adopted there is no desulfatation of galactose sulfate (b unit). After a while, the hydrolysis of the anhydrogalactose (a unit) produced D-galactose and 5-hydroxymethyl furfural ; as exemple after 148 hours the amount of D-galactose-4-sulfate ($1.27\ 10^{-3}$ mole) is nearly identic to the amount of D-galactose and furfural derivative ($0.43\ 10^{-3} + 0.92\ 10^{-3}$ mole), as obtained by preparative chromatography.

b) Enzymic hydrolysis. From viscosimetric measurement WEIGL and YAPHE (1966a) conclude to a random splitting of the chain with, at end, formation of a homologous series of oligomers $(ab)_n$. Up to now, no gel permeation chromatography of the series of oligomers formed has been published. Bio-Gel P2 and Sephadex-G 25 are not convenient supports due to large hydrodynamic volume of the sulfated oligosaccharides but Bio-Gel P6 provides excellent results. The oligomer elution volume Ve dependence (expressed by the distribution coefficient $K_d = \frac{V_e - V_o}{V_T - V_o}$) on the ionic strength of the eluent was determined (figure 3 and figure 4).

Over $5\ 10^{-2}$ M, the elution volumes remain unchanged. The limit of salt content was proposed previously as the minimum to screen electrostatic repulsion (RINAUDO et al. 1981). The degree of polymerisation n is determined by the Dubois' method and the Nelson's method. This attribution of index n is also confirmed ^{13}C NMR spectroscopy (ROCHAS and VINCENDON to be published). Whatever are the ionic strengths of the eluent, a linear relationship was found between Log K_d and DP over n = 1. This linear dependence agrees with the formation of an homologous series. The fraction (with n = 1,2,3,4,5) obtained by enzymic hydrolysis of the κ-carrageenan have been desalted on Bio-Gel P2 using water as eluent after preparative fractionation on Bio-Gel P6 in 0.05 M $NaNO_3$. Then the oligomers have been characterized by optical rotation, IR spectroscopy and ^{13}C NMR. These results are described in a following paper (ROCHAS and VINCENDON, to be published).

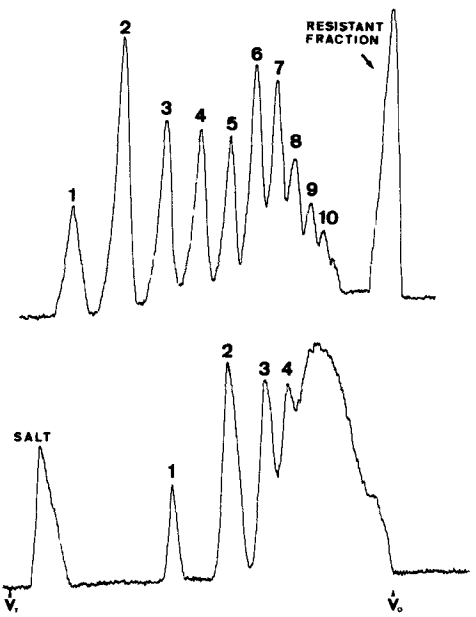

Fig. 3 : Fractionation of κ-carrageenan oligomers on Bio-Gel P6 column a) lower chromatogram eluent 10^{-3} M, b) upper chromatogram eluent 10^{-1} M.

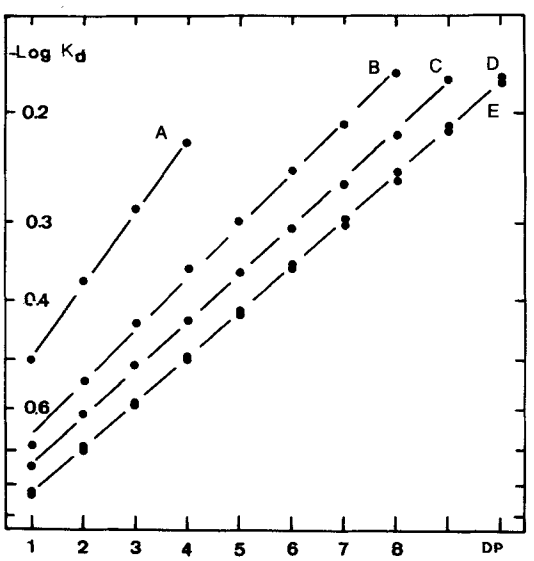

Fig. 4 : Relationship between DP of oligomers and Log K_d of κ-carrageenan oligomers with different eluents. A,B,C,D : 10^{-3}, 5 10^{-3}, 10^{-2}, 5 10^{-2}, 10^{-1} M $NaNO_3$.

For long time of hydrolysis and large amount of enzyme, the fraction of solute eluted on Vo is constant and around 11 % (W/W). The yield in sulfate group in this fraction is higher than the average value of the sample ($3.6\ 10^{-3}$ eq. g^{-1}, compared with $2.45\ 10^{-3}$ for κ-form), and the IR spectrum is similar to the iota-carrageenan IR spectrum. The intrinsic viscosity is 300 $ml.g^{-1}$ in 0.1 M NaCl at 25°C, when that of the initial κ-carrageenan is 770 ml g^{-1} with same conditions. This fraction of material rich in iota-carrageenan is not hydrolysed by the κ-carrageenase ; it is a high molecular weight polymer and it can be concluded that it represents at least large sequence of iota form in the fraction of molecules proposed as hybrid form. It is possible that a few units of κ-form at end of the iota sequence or κ-unit randomly distributed in the sequence are not cleaved by the enzyme and correspond to a resistant fraction with a proportion of 84% iota and 16% kappa (W/W) forms. Following, the net composition of the sample in pure iota unit should be 9 % corresponding to the yield found by ANDERSON et al. (1968, 1973), BELLION (1980) and BELLION et al. (1980).

A consequence of this structure is that at least 11 % W/W of the material is unaffected by κ-carrageenase and that the splitting mechanism cannot be described from viscosimetry as a random mechanism.

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