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ACID HYDROLYSIS OF THE POLYSACCHARIDE GUM FROM *Acacia podalyriaefolia* A. CUNN.: MOLECULAR-WEIGHT DISTRIBUTION STUDIES

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ABSTRACT

The course of acid hydrolysis of the polysaccharide gum of *Acacia podalyriaefolia* has been followed by gel chromatography. Elution patterns of samples removed at intervals demonstrate the rapid hydrolysis of arabinofuranoside linkages and subsequent, preferential removal of galactopyranose end-groups. The persistence of certain peaks in the hydrolysate elution-patterns may be explained on the basis of a galactan framework which could equally well be essentially linear or dendritically branched. The hydrolysis rate-constant decreases continuously with increasing degree of depolymerization.

INTRODUCTION

The occurrence of several peaks in the elution pattern obtained on gel chromatography of an hydrolysate (5mM sulphuric acid, 96°, 50 h) of the polysaccharide gum of *Acacia podalyriaefolia* has been noted previously[1,2]. The study presented here was undertaken in an attempt to account for this, to investigate the behaviour on acid hydrolysis of this and other arabinogalactans, and to seek further information on the structure of *A. podalyriaefolia* gum[2–4]. The molecular-weight distributions of degradation products of this polysaccharide are not complicated by polymolecularity of the parent polymer[1,2].

EXPERIMENTAL

The *A. podalyriaefolia* gum was a sample of the ethanol-precipitated material described earlier[3,4].

General methods. — Paper chromatography was carried out by the descending method on Whatman No. 1 paper with the solvent systems (v/v) (*a*) ethyl acetate–pyridine–water (10:4:3), (*b*) butyl alcohol–ethanol–water (1:1:1), and (*c*) butyl alcohol–ethanol–water (4:1:5, upper layer). Sugars were detected with *p*-anisidine hydrochloride in butyl alcohol[5].

Optical rotations were measured on a Bellingham and Stanley Model A polarimeter.

Reducing power was determined by the Nelson modification[6] of the Somogyi method[7], absorbances being measured at 546 nm on a Beckman DB spectrophotometer.

Gel chromatography. — Most of the hydrolysate samples were chromatographed on a column (90 × 1.5 cm) of the polyacrylamide gel Bio-Gel P-300 (Bio-Rad Laboratories). A column (55 × 1.2 cm) of the less porous P-10 gel was used for low molecular weight hydrolysates. Samples were applied in a volume of 1 ml in all cases. Where Bio-Gel P-300 was used, sample concentration varied from 2 to 8 mg/ml but, owing to the concentration-dependence[8] of polysaccharide elution volumes on Bio-Gel P-10, the concentration of samples chromatographed on this gel was kept within the range 2–3 mg/ml. *i.e.*, that employed in the calibration of the column[2]. Elution was carried out with M sodium chloride[9]. Fractions (1 ml) were assayed for carbohydrate by the phenol–sulphuric acid method[10], absorbances at 490 nm being measured on a Unicam SP600 spectrophotometer. Molecular weights corresponding to peaks in the elution curves were found from calibration plots as described previously[1,2].

Partial hydrolysis with 5 mM H_2SO_4. — *A. podalyriaefolia* gum (dry weight 1.88 g) was heated at 96° in 5 mM sulphuric acid (100 ml) for 96 h (pH rose from 2.1 to 2.4), samples (5 ml) being removed at intervals. Each hydrolysate sample was immediately cooled and its optical rotation measured. After neutralization (barium carbonate) and centrifugation, the samples were examined by chromatography on paper and on Bio-Gel P-300. The reducing power of each hydrolysate was determined in duplicate, 1-ml aliquots being diluted so that the concentration of reducing sugar lay within the range 25–250 μg/ml. The degree of scission (x) of the polysaccharide in each case was calculated by dividing the reducing power (expressed as g of reducing sugar/g of polysaccharide) in excess of that of the gum itself by that of a sample of the gum subjected to prolonged hydrolysis (0.5M sulphuric acid, 96°, 18 h).

Further hydrolysis with 50 mM H_2SO_4. — The acid concentration in the solution (15 ml) remaining after the treatment with 5 mM sulphuric acid was adjusted to 50 mM by the addition of 0.5M sulphuric acid (*ca.* 2 ml), and the solution was heated for a further 20 h at 96° (pH 1.1 to 1.3). Samples (3 ml) removed at intervals were examined as described above, except that the hydrolysates obtained after 8 h or more were chromatographed on Bio-Gel P-10.

RESULTS AND DISCUSSION

Neutral sugar components of hydrolysates. — The only sugar detected on paper chromatography (solvents *a*, *b*, and *c*) of the hydrolysates obtained after treatment of the gum with 5 mM sulphuric acid for 8 h or less was arabinose. Galactose and a trace of rhamnose were found after 12 h, but oligosaccharides only after 24 h. However, the response to the spray reagent of a series of sugars [D-galactose, 6-*O*-β-D-galactopyranosyl-D-galactose, and the corresponding β-D-(1→6)-linked trisaccharide], present in a mixture in equal amounts, was found to decrease markedly with increasing degree of polymerisation. It is possible, therefore, that oligosaccharides, in amounts not detectable by paper chromatography, are produced at an earlier stage of hydrolysis.

The use of solvent *a* permitted the resolution of several oligosaccharide components in the hydrolysates obtained on treatment of the gum with 5 mM sulphuric acid for 24 to 96 h. Two disaccharides, identified as 3- and 6-*O*-β-D-galactopyranosyl-D-galactose on the basis of their chromatographic mobility (R_F 0.42 and 0.35, respectively), were detected, and five higher oligosaccharides (R_F 0.28, 0.17, 0.12, 0.08, and 0.04) were also resolved. The linearity of a plot of the values of log $[(1/R_F)-1]$ for these components and for D-galactose and the β-(1→3)-linked D-galactose disaccharide against the suspected degree of polymerisation (hexose units) is consistent[11] with the behaviour of a homologous series of β-(1→3)-linked D-galactose oligosaccharides.

This series of oligosaccharides was also detected (paper chromatography, solvent *a*) in the hydrolysates obtained on further treatment of the degraded gum with 50 mM sulphuric acid for 8 h or less. After 20 h, sugars higher than the trisaccharide were no longer present in detectable amounts.

Molecular-weight distributions. — The elution patterns obtained on gel chromatography of the hydrolysate samples are shown in Figs. 1–3. The values of $\overline{M}_w$, the weight-average molecular weight[12], calculated in each case from the molecular weights corresponding to peaks in the elution curve and the relative areas beneath these peaks, are given in Tables I and II, together with the specific rotations and values of the degree of scission, x, for the various hydrolysates.

The elution patterns of samples withdrawn during the initial stages of hydrolysis (Fig. 1, *A–C*) reflect the usual, rapid hydrolysis of the acid-labile arabinofuranoside linkages[13,14] in the gum. Since these linkages are almost exclusively peripheral in the *A. podalyriaefolia* gum polysaccharide[4], the initial decrease in $\overline{M}_w$ with increasing x is small. Calculations based on the known[3] arabinose content of the gum (mole % 16) predict a molecular weight of 27,200 after hydrolysis of all arabinofuranoside linkages; this agrees with the values (27,500 and 26,500) estimated from the elution volumes of the second polysaccharide peak in *B* and *C* (Fig. 1), respectively.

A striking change in the gel-chromatography elution-pattern occurs after treatment of the gum with 5 mM sulphuric acid for 12 h (Fig. 1, *D*). The peak corresponding to a molecular weight of 31,000 (largely undegraded gum) disappears, that at molecular weight 26,500 is considerably attenuated, and a large peak appears at molecular weight 17,800.

The molecular weight of the product obtained after removal of arabinose and all galactopyranose units present as end-groups or in short branches (3 units or less) in the *A. podalyriaefolia* gum polysaccharide may be estimated on the basis of the degree of branching of the molecule, determined by methylation analysis[3]. Calculations based on a dendritically branched model of the galactan framework give a value of 17,600, while the use of a model consisting of an essentially linear chain with multiple short-branches predicts a similar value, agreeing well with the molecular weight (17,800) corresponding to the large peak in Fig. 1, *D*. The occurrence of this peak is therefore consistent with the preferential hydrolysis of terminal galactopyranose residues, and those in short branches which rapidly become

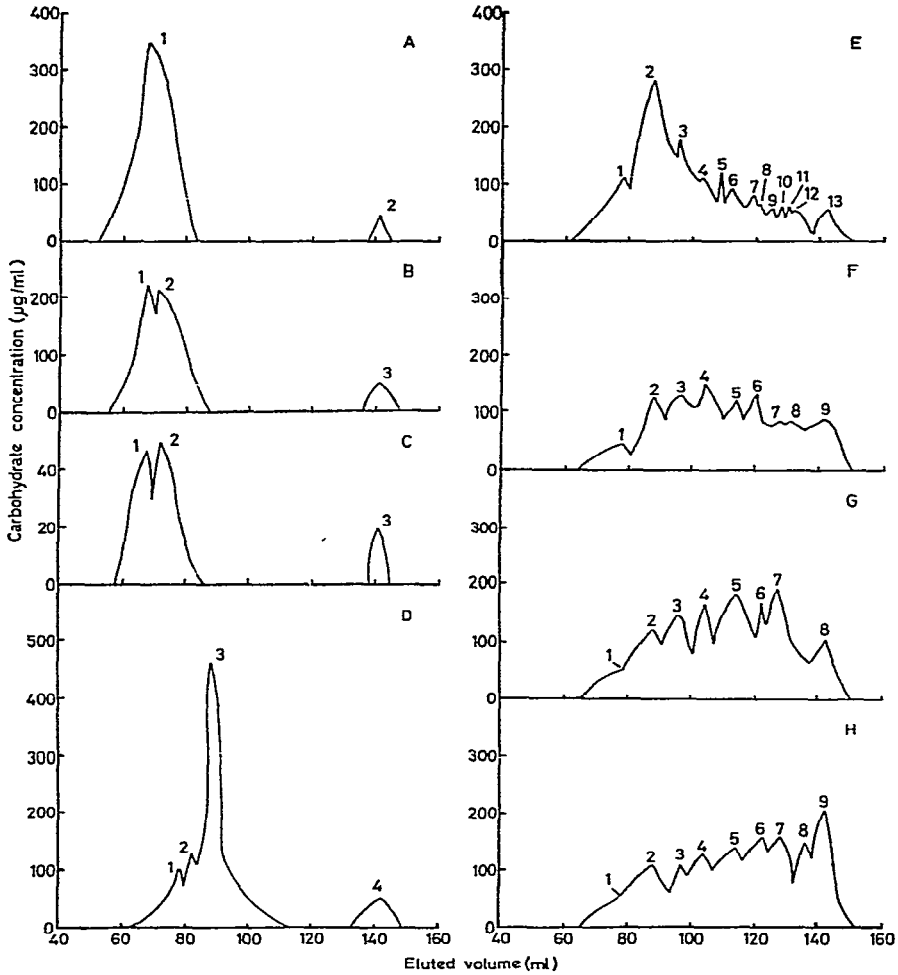

Fig. 1. Bio-Gel P-300 elution patterns (*A–H*) of hydrolysates of gum treated with 5 mM sulphuric acid at 96°. For each, there follow duration of hydrolysis and molecular weights corresponding to peaks as numbered: *A*. 2 h: 1, 31,000; 2, ⩽1,800. *B*. 5 h: 1, 31,000; 2, 27,500; 3, ⩽1,800. *C*. 8 h: 1, 31,000; 2, 26,500; 3, ⩽1,800. *D*. 12 h: 1, 26,500; 2, 22,400; 3, 17,800; 4, ⩽1,800. *E*. 24 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 10,000; 5, 7,800; 6, 6,700; 7, 5,300; 8, 4,900; 9, 4,200; 10, 3,700; 11, 3,400; 12, 3,200; 13, ⩽1,800. *F*. 48 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 9,400; 5, 6,500; 6, 5,000; 7, 3,800; 8, 3,300; 9, ⩽1,800. *G*. 72 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 9,400; 5, 6,500; 7, 3,800; 8, ⩽1,800. *H*. 96 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 9,400; 5, 6,500; 6, 4,500; 7, 3,700; 8, 2,700; 9, ⩽1,800.

terminal after such hydrolysis. The now considerable body of evidence in favour of the view that acid hydrolysis of terminal linkages in polysaccharides is more rapid than that of internal bonds has recently been reviewed by BeMiller[15].

The elution pattern obtained after treatment of the gum with 5 mM sulphuric acid for 24 h (Fig. 1, *E*) shows, in addition to the peaks at molecular weights 26,500 and 17,800, which have decreased in magnitude, several small peaks at elution volumes

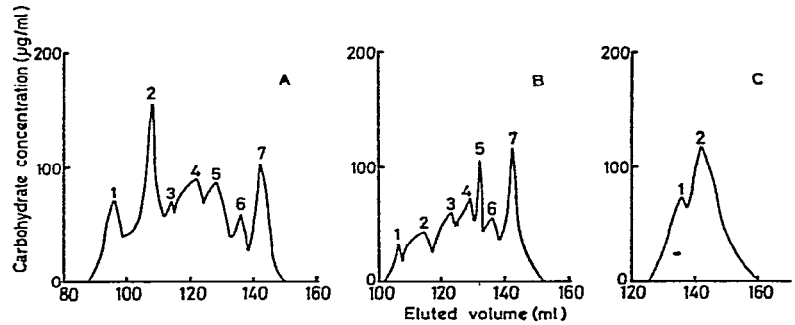

Fig. 2. Bio-Gel P-300 elution patterns (*A–C*) of hydrolysates obtained on treatment of partially hydrolysed gum with 50 mM sulphuric acid at 96°. *A*. 1 h: 1, 13,000; 2, 8,400; 3, 6,500; 4, 4,700; 5, 3,700; 6, 2,700; 7, ⩽1,800. *B*. 3 h: 1, 8,800; 2, 6,500; 3, 4,500; 4, 3,500; 5, 3,200; 6, 2,700; 7, ⩽1,800. *C*. 8 h: 1, 2,700; 2, ⩽1,800.

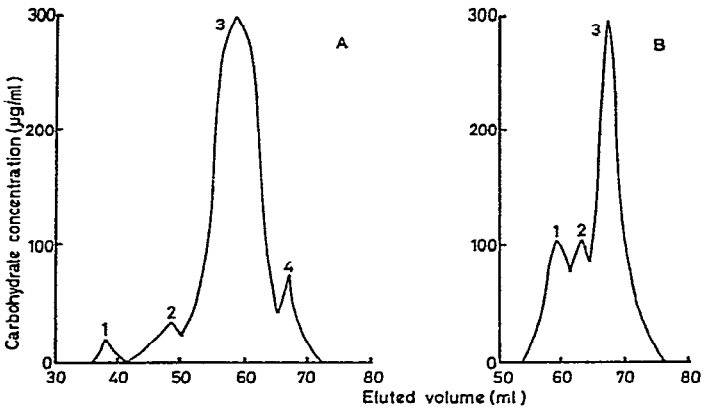

Fig. 3. Bio-Gel P-10 elution patterns (*A*, *B*) of hydrolysates obtained on treatment of partially hydrolysed gum with 50 mM sulphuric acid at 96°. *A*. 8 h: 1, 2,700; 2, 1,300; 3, 500; 4, ⩽250. *B*. 20 h: 1, 490; 2, 350; 3, ⩽250.

TABLE I

PARTIAL HYDROLYSIS OF *A. podalyriaefolia* GUM BY 5mM SULPHURIC ACID

Time of hydrolysis (h)	*Degree of scission, x*	$[\alpha]_D^{20}$ *(degrees)*[a]	$\overline{M}_w$	10^6 k (sec^{-1})
0	—	+5.5	31,500 (Ref. 1)	
2	0.009	+6.9	31,000	1.25
5	0.022	+12	29,000	1.24
8	0.033	+16	28,200	1.04
12	0.046	+21	19,800	0.944
24	0.077	+31	14,800	0.762
48	0.112	+37	10,600	0.421
72	0.136	+40	10,000	0.344
96	0.156	+41	9,100	0.272

[a]In 5mM sulphuric acid (*c* 1.88).

TABLE II

FURTHER HYDROLYSIS OF GUM BY 50mM SULPHURIC ACID

Time of further hydrolysis (h)	*Degree of scission, x*	$[\alpha]_D^{20}$ *(degrees)*[a]	$\overline{M}_w$	10^6 k (sec^{-1})
1	0.359	+48	5,700	
3	0.500	+54	3,500	34.5
8	0.660	+61	550	26.9
20	0.786	+72	280	8.44

[a]In 50mM sulphuric acid (*c* 1.66).

corresponding to lower molecular weights. As the time of hydrolysis increases from 24 to 96 h, peaks at molecular weights of *ca.* 26,500, 17,800, 13,000, 9,400, 6,500, 4,700, and 3,800, as well as the sugar peak ($\leq$1,800), persist in the elution patterns (Fig. 1, *F–H*), the low molecular-weight peaks growing at the expense of those corresponding to higher molecular weights with increasing degree of hydrolysis. On further treatment of the degraded gum with 50mM sulphuric acid, the polysaccharide peaks gradually disappear (Fig. 2); chromatography on Bio-Gel P-10 shows the hydrolysates obtained after 8 h or more to consist of mixtures of sugars (Fig. 3).

Apart from the values of 26,500 and 17,800, which have been discussed, the molecular weights corresponding to the persistent peaks in the hydrolysate elution-patterns are all approximately half of the values associated with peaks at lower elution volumes (*e.g.* the series 26,500, 13,000, 6,500). It is possible, therefore, that the occurrence of several peaks, rather than a single, broad peak at a molecular weight corresponding to $\overline{M}_w$ for the appropriate degraded polysaccharide, is simply a reflection of the fact that, on scission of a middle linkage in a chain, two fragments of equal size are produced by breaking only one bond. High yields of such fragments may thus be expected at the appropriate degree of scission[16,17].

Various statistical treatments of the depolymerization of long-chain molecules have been published[16–19]. Montroll and Simha[17] considered the decrease of $\overline{M}_w$ with increasing degree of scission, x, in the case of completely random depolymerization and derived the equation

$$\frac{\overline{M}_w}{M_o} = \frac{nx^2 + 2(1-x)[(1-x)^n + nx - 1]}{nx^2}, \qquad (1)$$

where M_0 = molecular weight of monomer unit, and n = number of monomer units in undegraded polymer.

Simha[18] subsequently considered the case of preferential scission of terminal linkages, the rate constant for the breaking of internal linkages being regarded as negligible in comparison with that for terminal bonds; thermal depolymerization of polystyrene in the liquid phase at 350° was cited as an example of a reaction believed

to proceed in this way. For this situation, he derived the equation

$$\frac{\bar{M}_w}{M_o} = 1+e^{-z}\left[(n-1)\frac{z^{n-2}}{(n-2)!}+\left(n-2z-1+\frac{2z}{n}\right)\frac{z^{n-3}}{(n-3)!}\right.$$

$$\left.+\left(1-\frac{z}{n}\right)\left(n-z-1+\frac{z}{n-z}\right)\left(e^{z}-\sum_{r=n-3}^{\infty}\frac{z^r}{r!}\right)\right]+\frac{2R}{n}, \qquad (2)$$

where $z = 2kt$, k being the hydrolysis rate-constant at time t,

$$R = e^{-z}2^{n-2}\sum_{i=n-1}^{\infty}\left(\frac{z}{2}\right)^i\frac{1}{i!},$$

and M_o and n have the same significance as in equation (1).

The variation of $\bar{M}_w/M_o$ with x on progressive acid-degradation of the *A. podalyriaefolia* gum polysaccharide, as determined in the present work*, is shown in Fig. 4 (curve 1). Fig. 4 (curve 2), which would be obtained if hydrolysis were almost exclusively confined to terminal linkages in the initial stages ($x \leq 0.156$), has been calculated from equation (2), the value of z given by the values of k (see below) and t at the appropriate x (Table I) being inserted into the equation in each case. Fig. 4 (curve 3), calculated from equation (1), shows the $\bar{M}_w/M_o$ values predicted if depolymerization were completely random after hydrolysis of the arabinofuranoside linkages was essentially complete. In this case, the molecular weight of the parent polymer was taken as 27,200 (the theoretical value for the product obtained after removal of all arabinose from the gum), and the x values inserted into equation (1) were calculated with the value (0.038, from Fig. 4, curve 1) corresponding to this $\bar{M}_w$ as the origin.

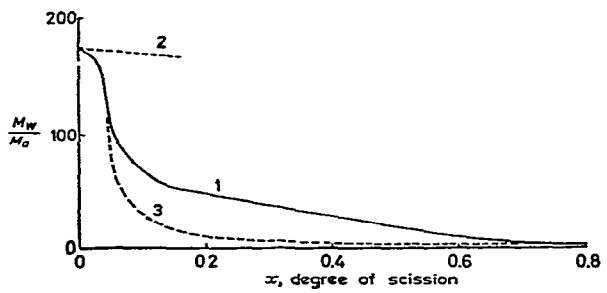

Fig. 4. Variation of $\bar{M}_w/M_o$ with degree of scission. 1. Experimental values. 2. Values predicted by equation (2). 3. Values predicted by equation (1).

It is evident from Fig. 4 that the experimentally determined values of $\bar{M}_w/M_o$ deviate from curve 2 to an increasing extent as x increases and hydrolysis of peripheral arabinofuranoside linkages, approaching completion, has less dominance in the

*$\bar{M}_w/M_o$ has been calculated on the basis of a value of 180 for M_o. Allowance for varying proportions of arabinose in the polysaccharide has a negligible effect upon the calculated values of $\bar{M}_w/M_o$, owing to the low arabinose content of the gum.

overall hydrolysis. However, the experimental curve lies above curve 3 over most of its length; the two curves coincide only at high x (≥ 0.74). Hydrolysis of this polysaccharide is clearly non-random, even after cleavage of most of the arabinofuranoside linkages. Reasons for this include the faster hydrolysis of terminal than internal galactopyranose residues, as suggested above, and the presence of both β-(1→3) and β-(1→6) linkages between D-galactopyranose residues[2,3]. The hydrolysis rates of these linkages in the D-galactobioses are appreciably different[20] [β-(1→3) faster than β-(1→6)], and differences might therefore be expected in the case of the polysaccharide also. Furthermore, both chain units and branch-points are present in comparable amounts[2].

Kinetics of hydrolysis. — The mean values of the hydrolysis rate-constant, k, over the successive time-intervals between samples are given in the extreme right-hand columns of Tables I and II. These values were calculated from the equation

$$k = \frac{1}{t_2 - t_1} \ln \frac{1 - x_1}{1 - x_2},$$

where t_1 and t_2 are the times of hydrolysis corresponding to consecutive measured values, x_1 and x_2, of the degree of scission.

It is clear that, under given conditions, k decreases with increasing x. This is in contrast to the acid hydrolysis of cellulose[21] and amylose[22], in which an increase in the rate constant, attributed to increasing proportion of end-groups, is observed as hydrolysis progresses. In the present work, the effect of any increase in the proportion of end-groups will be small in comparison with the decrease in hydrolysis rate which must occur as the rapid hydrolysis of arabinofuranoside linkages approaches completion and the overall rate of hydrolysis becomes increasingly dependent upon the slower disintegration of the galactan framework of the polysaccharide.

The relative magnitudes of these two effects may be assessed by comparing rate constants given in the literature. Myrbäck and Magnusson[23] have calculated that the end-groups in starch are hydrolysed 1.68 times more rapidly than the internal linkages; in the case of cellulose, a ratio of *ca.* 3:1 has been estimated[21]. In contrast, comparison of values reported for the hydrolysis constants of various methyl glycosides[15,24] shows that methyl β-D-galactopyranoside is hydrolysed 17 times more slowly than methyl α-L-arabinofuranoside under the same conditions. (The initial increase in specific rotation on acid hydrolysis of *A. podalyriaefolia* gum is consistent with the presence of α-L-arabinofuranoside linkages.) The value found for k in the early stages of the treatment of the gum with 5mM sulphuric acid (1.25×10^{-6} sec^{-1}, from Table I) is, however, much lower than that reported[24] for the hydrolysis of methyl α-L-arabinofuranoside in 5mM acid ,at 100° (260×10^{-6} sec^{-1})*. Furthermore, the decrease in k during 96 h is less than expected from the values of k for the respective methyl glycosides[25]. Hydrolysis rates of linkages in polysaccharides are not strictly comparable with those of the same linkages in simple glycosides, owing to

*The value given in the reference cited is based on common logarithms; here it has been converted into that based on natural logarithms, in accordance with current practice[15].

steric and other factors[15]. The rate of hydrolysis of the arabinofuranoside linkages, however, remains higher than that of galactopyranoside. Moreover, in the case of a molecule as highly branched as the *A. podalyriaefolia* gum polysaccharide[3], the proportion of end-groups will not necessarily increase with decreasing degree of polymerization; with the removal of short side-chains as hydrolysis progresses, it is more likely to decrease. The continuous decrease in hydrolysis rate-constant with increasing degree of scission of the polysaccharide, when the hydrolysis conditions remain constant, is therefore not unexpected.

The values found for k on hydrolysis of the partially degraded polysaccharide by 50mM acid (Table II) are all considerably lower than those for hydrolysis of the D-galactobioses under comparable conditions [*ca.* 200×10^{-6} and 80×10^{-6} sec^{-1} for the β-(1→3) and β-(1→6) linked isomers, respectively, from the half-hydrolysis times[20]]. This may be attributed to the retarded hydrolysis of inner bonds in the galactan framework; cleavage of the peripheral linkages possibly proceeds at a rate comparable with that of hydrolysis of the bioses[15], except at branch-points[26].

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