MARINE PLANT POLYMERS

PART III*. A KINETIC ANALYSIS OF THE ALKALINE DEGRADATION OF POLYSACCHARIDES WITH SPECIFIC REFERENCE TO (1→3)-β-D-GLUCANS†

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ABSTRACT

A general kinetic expression is presented for the rate of alkaline degradation of linear polysaccharides in terms of mono- and di-anionic species formed from the reducing end-groups. Specific rate constants have been determined for the end-wise depolymerization of the (1→3)-β-D-glucans, laminaran, laricinan, and pachyman, and compared with similar data for amylose degradation. The rate constants of degradative chain-propagation via the mono- and di-anion intermediates have been shown to be essentially equal. The effect of the type and concentration of base on the mechanism of end-wise degradation is described for both (1→3)-linked and (1→4)-linked polysaccharides. Inhibition of alkaline degradation is discussed in terms of chain branching and blockage of reducing end-groups.

INTRODUCTION

The alkaline lability of (1→3)-β-D-glucans has been observed during investigations of the properties and utilization of marine polymers[1]. (1→3)-β-D-Glucans are widely distributed in Nature[2,3], occurring within the marine environment as leucosin or chrysolaminaran in diatoms[4,5], as paramylon in the Euglenophyta[6], and as laminaran in the Phaeophyta[7]. They also are often substantial constituents of marine particulate matter[8].

The Phaeophycean product, laminaran, has been extensively investigated and is therefore the best defined of this class of polymers. Two less well defined but readily available terrestrial (1→3)-β-D-glucans are pachyman, from the fungus *Poria cocos*[9], and laricinan, which has recently been isolated from the cell wall of *Larix laricina* compression wood by Hoffman and Timell[3]. These three materials were therefore selected as typical representatives in a kinetic examination of the alkaline degradation of (1→3)-linked polysaccharides.

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†Part of the Ph. D. research of R. A. Young.

Although the mechanisms of alkaline degradation of (1→3)- and (1→4)-linked polysaccharides have been extensively clarified[10], kinetic data are available only for the latter polymers[11,12]. Both the (1→3)-linked[13] and (1→4)-linked polymers undergo degradation by β-alkoxy elimination via an enolate ion intermediate. A notable difference, however, is the occurrence of a termination or "stopping" reaction in (1→4)-linked polysaccharides that involves end-group conversion into a metasaccharinic acid moiety[11,14,15]. Since no analogous mechanism of termination is known for the degradation of linear (1→3)-linked polysaccharides, these polymers should exhibit the total end-wise depolymerization illustrated by the sequence of reactions in Fig. 1. Nonetheless, inhibition of total degradation can result from (*a*) modification

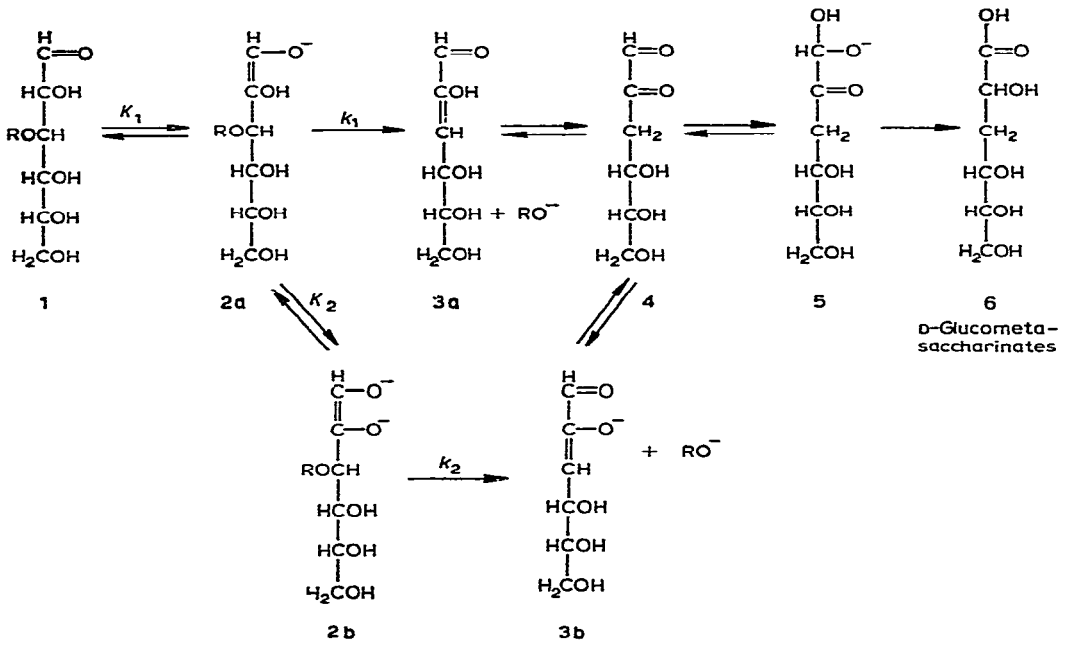

Fig. 1. Mechanism of the alkaline degradation of a (1→3)-linked polysaccharide illustrated for a reducing end-group; R represents the rest of the polysaccharide molecule.

of end-groups during isolation, as exemplified by oxidation to aldonic acid groups, (*b*) blockage of the reducing end-groups through the presence of glycosidic alditol or phenol ether bonds, and or (*c*) branching. For example, the endwise depolymerization of laminaran[13] ceases after a polysaccharide weight-loss of only 40%. This inhibition of total degradation was explained first, by the presence of end-units blocked by glycosidically linked mannitol in a proportion (40–46%) of the laminaran chains (M-chains)[7], and secondly, by the occurrence of a small proportion of (1→6)-linkages in the chains which are not terminated with a mannitol unit (G-chains). Branching of

this type generates stable oligomers having glucometasaccharinic acid end-groups (Fig. 2). Alditol end-groups seem to be absent in pachyman and laricinan, the other two materials used in this study, although branching may occur to some extent in both cases.

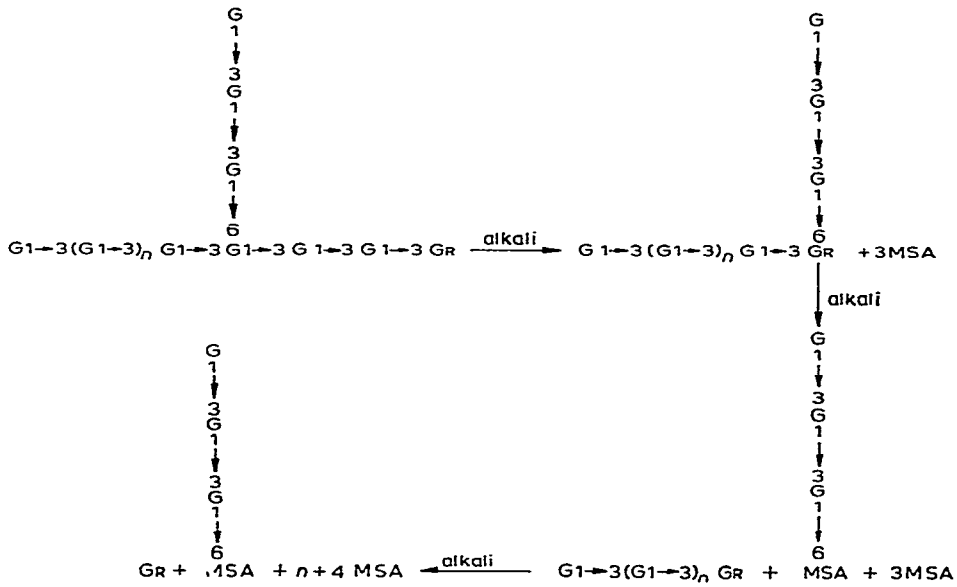

Fig. 2. Effect of C-6 branches on the degradation of (1→3)-glucans. G, GR and MSA represent D-glucose-, reducing end-, and glucometasaccharinic acid residues, respectively.

THEORY

The probable reaction sequence leading to end-group elimination of reducing polysaccharides can be represented by the scheme illustrated in eqn. (*1*). This representation is supported by the results obtained from the alkaline degradation of amylose[11] and may be valid for base-catalyzed degradations of polysaccharides in general. A more detailed reaction sequence is shown for (1→3)-linked polysaccharides in Fig. 1. (1→4)-Linked polysaccharides undergo, of course, end-group rearrangement to ketose groups prior to the formation of the relevant anionic species.

$$\text{(Polys.)-Gr} \overset{K_1}{\rightleftarrows} \text{(Polys.)-Gr}^- + \text{H}^+ \overset{K_2}{\leftrightarrows} \text{(Polys.)-Gr}^{2-} + \text{H}^+ \qquad (1)$$

k_1 → Endwise degradation; k_2 ← ; k_3 → Endgroup stabilization (chain termination)

In eqn. (*1*) it is presumed that the reaction occurs only via the anionic species, in this case the mono- and di-anionic forms of the end-group[16,17]. Thus, in alkaline solutions, it is likely that an equilibrium is approached between neutral, mono- and

di-anionic end-group species, characterized by the equilibrium constants K_1 and K_2. Both mono- and di-anions are reactive towards peeling, whereas end-group stabilization, which occurs in (1→4)-linked polymers only, is achieved via the di-anionic species by conversion into a metasaccharinic acid end-group[11].

For an interpretation of the foregoing scheme (eqn. *1*), the assumption is made that the reaction sequence from the anionic species to the peeled-off unit can be adequately represented by one rate constant, such as k_1, rather than by a series of rate constants representing each transformation (*e.g.* glucose→fructose) as determined by MacLaurin and Green[18] for a number of sugars and disaccharides. This assumption is implicit in previous kinetic derivations for the alkaline degradation of polysaccharides[11,12].

From the reaction scheme (eqn. *1*), degradative chain-propagation and chain-termination are expressed by equations (*2a*) and (*2b*).

$$\mathrm{d}[Ge]/\mathrm{d}t = k_1[Gr^-] + k_2[Gr^{2-}] = \mathrm{d}L/\mathrm{d}t \qquad (2a)$$

$$-\mathrm{d}[Gr]/\mathrm{d}t = k_3[Gr^{2-}] \qquad (2b)$$

where t represents the reaction time; L, the weight fraction of polysaccharide degraded after time t; $[Ge]$, the mole fraction of peeled-off end-groups at time t; $[Gr]$, the total mole fraction of remaining end-groups at time t; $[Gr^-]$ and $[Gr^{2-}]$, the mole fractions of relevant mono- and di-ionized end-groups; while k_1, k_2, and k_3 are the rate constants for the reactions depicted in the scheme (eqn. *1*).

From reaction sequence (*1*) it follows that the first and second ionization constants, K_1 and K_2, can be represented by:

$$K_1 = [Gr^-][H^+]/\{[Gr] - ([Gr^-] + [Gr^{2-}])\} \qquad (3)$$

and $K_2 = [Gr^{2-}][\mathrm{H}^+]/[Gr^-]$, respectively. (4)

Elimination of the term $[\mathrm{Gr}^{2-}]$ from eqn. (*3*) by using eqn. (*4*) shows that

$$[Gr^-] = K_1[Gr][\mathrm{H}^+]/([\mathrm{H}^+]^2 + K_1[\mathrm{H}^+] + K_1K_2). \qquad (5)$$

Similar elimination of the term $[Gr^-]$ from eqn. (*4*) by using eqn. (*5*) indicates that $[Gr^{2-}] = K_1K_2[Gr]/([\mathrm{H}^+]^2 + K_1[\mathrm{H}^+] + K_1K_2)$. (6)

Combination of eqns. (*5*), (*6*), and (*2*) yields the rate expression

$$\mathrm{d}L/\mathrm{d}t = [Gr]\{(k_1K_1[\mathrm{H}^+] + k_2K_1K_2)/([\mathrm{H}^+]^2 + K_1[\mathrm{H}^+] + K_1K_2)\}. \qquad (7)$$

Then if $s = K_1/([\mathrm{H}^+]^2 + K_1[\mathrm{H}^+] + K_1K_2)$, eqn. (*7*) becomes

$$\mathrm{d}L/\mathrm{d}t = s[Gr](k_1[\mathrm{H}^+] + k_2K_2). \qquad (8)$$

Thus, from eqns. (*6*) and (*2*), the rate of chain termination can be written as $-\mathrm{d}[Gr]/\mathrm{d}t = sk_3K_2[Gr]$ which, on integration, becomes

$$[Gr] = [Gr]_0 \exp(-sk_3K_2t), \qquad (9)$$

where $[Gr]_0$ represents the mole fraction of end groups at zero time, and hence from eqns. (*8*) and (*9*)

$$dL/dt = s(k_1[H^+]+k_2K_2)[Gr]_0 \exp(-sk_3K_2t), \quad (10)$$

which is a general expression for the rate of alkaline degradation of linear polysaccharides. Evaluation of eqn. (*10*) by integration demonstrates that

$$L = \{s(k_1[H^+]+k_2K_2)[Gr]_0\}\{1\text{-}\exp(-sk_3K_2t)\}/sk_3K_2 \quad (11)$$

and so at infinite time

$$L_\infty = [Gr]_0(k_1[H^+]+k_2K_2)/k_3K_2, \quad (12)$$

where L_∞ is the weight fraction of polysaccharide degraded after an infinite time. For (1→3)-linked linear polysaccharides, $k_3 = 0$, $L_\infty = 1$, $[Gr] \simeq [Gr]_0$, and eqn. (*10*) becomes

$$dL/dt = s(k_1[H^+]+k_2K_2)[Gr]_0. \quad (13)$$

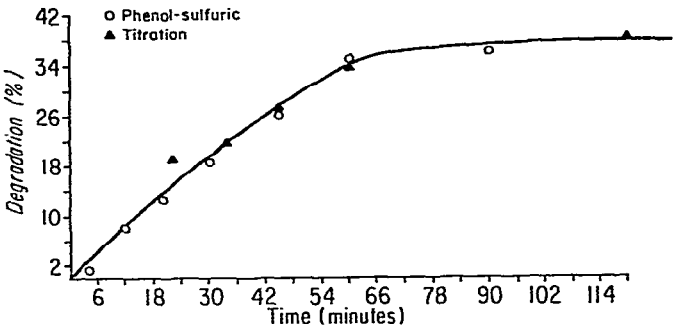

Fig. 3. Degradation of laminaran in 0.1M NaOH at 56°, monitored by two separate methods.

DISCUSSION AND RESULTS

Fig. 3 illustrates the percent of laminaran (water-insoluble form) degraded as a function of time in 0.1M NaOH at 56°. The extent of degradation was determined by two complementary methods; titrimetry and the phenol–sulfuric acid reaction[19]. The phenol–sulfuric acid reaction, which measures the total residual undegraded carbohydrate, has been used for the analysis of alkaline degradation of disaccharides[20,21]. Moreover, Lindberg *et al.*[21] demonstrated that, in these cases, the saccharinic acid products did not interfere with the color reaction. The titrimetric method detects the acids formed from peeled-off units. The ratio of one equivalent of acid per mole of peeled-off D-glucose was established, in full agreement with the work of Corbett and Kenner[13] where it was found that laminaran was degraded predominantly to glucometasaccharinic acids in oxygen-free lime-water. Because of convenience of application and the small sample size necessary, the phenol–sulfuric acid reaction was used for the analysis in the majority of the kinetic runs.

The effect of alkali concentration on the degradation of laminaran at 56° is shown in Fig. 4. The experimental rate-constants were obtained from these plots.

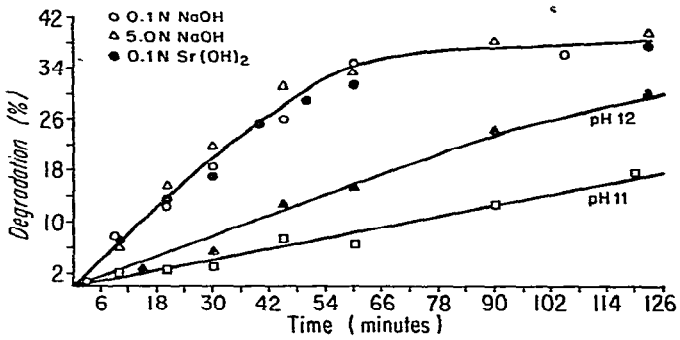

Fig. 4. Effect of hydroxide-ion concentration and base on the extent of degradation of laminaran at 56°.

TABLE I

DISSOCIATION CONSTANTS FOR GLUCOSE AND GLUCANS*

Sample	*Temp (°C)*	$K_1 \times 10^{13}$	$K_2 \times 10^{14}$	*Reference*
D-Glucose	25	5.25		22
	25	7.76–8.13	1.41–1.50	16, 23, 24
α-D-Glucose	25	3.45		26
β-D-Glucose	25	6.75		26
Amylose (reducing end-groups)	100		2.0	11

*For the over-all dissociation constants of amylose and cellulose, see ref. 27 and 28.

At one-half the maximum rate for degradation of laminaran, the pK_1 value for the first ionization to monoanion was determined as 12.2. This number is in good agreement with pK_a values determined by previous investigators (Table 1) for formation of a monoanion with both D-glucose and glucans. For D-glucose and amylose, the pK_2 value for the dissociation to dianions is about 13.8 (Table 1). The second dissociation-constant for laminaran end-groups could not be estimated from the experimental data, but is expected to be of similar magnitude.

The experimentally determined rates for degradation of laminaran at 56° are plotted in Fig. 5 and are compared with a theoretical plot derived from eqn. (*13*). The approximate magnitudes of the dissociation constants K_1 and K_2 were obtained from the foregoing pK values. These empirical data were employed to estimate the rate constants k_1 and k_2. Since there was no increase in the rate in the upper pH range where the dianionic species is the major reaction intermediate, it was concluded that the rate of β-elimination from both mono- and di-anions was essentially the same. Thus, the rate constants k_1 and k_2 must be nearly equal, and were calculated for a single value of the experimental rate at 56°. The satisfactory agreement between the experimental points and the theoretical curve confirms this assumption. The equality of k_1 and k_2 is probably also valid for most (1→4)-linked polysaccharides, since the

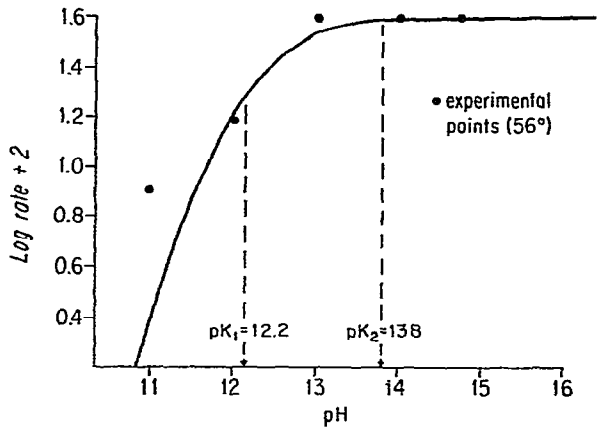

Fig. 5. Comparison of experimental rate-data with a curve derived from the following assigned values: $k_1 = k_2 = 10.9\ h^{-1}$, $K_1 = 6.76 \times 10^{-13}$, and $K_2 = 1.58 \times 10^{-14}$.

rate of degradative propagation of amylose shows[11], like laminaran, no inflection in the rate plot above a pH of approximately 13.

The situation for (1→4)-linked polysaccharides is different, however, since the additional effect of end-group stabilization via the dianion must be taken into account, as illustrated in reaction sequence (*1*) and eqn. (*9*). As a result of this "stopping" reaction, the extent of degradation is dependent on the concentrations of alkali. Above pH values of 13, the polymer will be degraded at a constant initial rate but, as a result of enhanced termination, the maximum extent of degradation (L_∞) will decrease with increasing alkalinity. However, at lower alkali concentrations (less than 0.1M), where the monoanion is the predominant species, the termination process may be insignificant, allowing the peeling process to proceed to completion[11].

It should be noted at this point that the termination reaction is initiated by β-elimination of a hydroxide ion (from C-3), in contrast to the degradation reaction in which the leaving β-group is a glycosyloxy anion. The latter is a better leaving group, which explains why the elimination process is possible for a monoanionic species. The release of a hydroxide ion requires, apparently, additional coulombic assistance from a second anionic group in the molecule. Similar effects have been observed in the reaction of D-glucose with alkali[29], where the degradation to gluco-metasaccharinic acid requires a high enough alkalinity (~8N) to convert the D-glucose to its dianionic form. Whereas the mechanism of termination finds a satisfactory explanation from this rationale, the lack of a difference between the rates of elimination of glucoside anions from mono- and di-anionic end-group species still remains obscure.

For comparison of the rate constants (k_1 and k_2) for laminaran and amylose it is important to realize that these constants are associated with slightly different steps for (1→3)-linked and (1→4)-linked polysaccharides. In Fig. 1, which represents the currently accepted mechanism of alkaline degradation of (1→3)-linked polysac-

charides[10,13,17,30–32], it can be seen that the β-elimination occurs directly to yield the unsaturated species (**3ab**). The end-groups of (1→4)-linked polysaccharides, in contrast, must rearrange to the ketose form before β-elimination can take place. Previous work on disaccharides[18,33] suggests that the rate of formation of the ketose intermediate may be comparable to the rate of the β-elimination step. As a consequence, the rate of the actual β-elimination must be faster than the observed rate of degradation, represented by the constants k_1 and k_2. In spite of such differences in the mechanisms of degradation for (1→3)- and (1→4)-linked polysaccharides, the calculated rate-constants differ by only a factor of two. For example, at 56°, $k_1 = k_2 = 10.9\ h^{-1}$ for the (1→3)-linked laminaran, whereas $k_1 = k_2 = 5.95\ h^{-1}$ for the (1→4)-linked amylose (Table II).

TABLE II

CALCULATED VALUES OF RATE CONSTANTS FOR GLUCANS DEGRADED IN SODIUM HYDROXIDE SOLUTIONS

Glucan	*Temp.* (°C)	*NaOH concentration* (M)	L_∞	*D.p.*	$k_1 = k_2\ h^{-1}$	$k_3\ h^{-1}$
Amylose	56	1.25	0.21	820	5.95	0.53
	100	0.1			323	
		1.0	0.45		298	1.33
Laminaran	78	0.1	0.42	25	74.2	
	56				10.9	
	40				2.10	
Laricinan	56	0.1	0.30	165	11.5	
Pachyman	56	0.1	0.45	700	29.6	

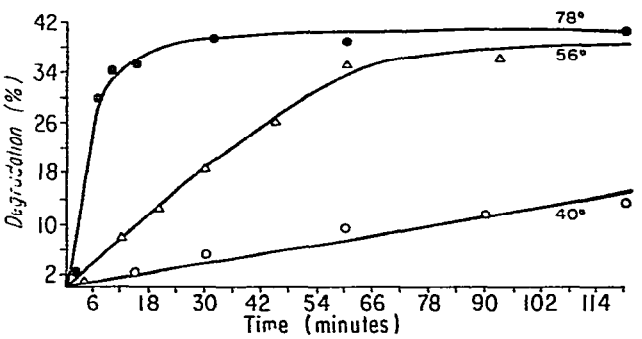

Fig. 6. Effect of temperature on the degradation of laminaran in 0.1M NaOH.

The effect of temperature on the rate of degradation of laminaran in 0.1M sodium hydroxide is shown in Fig. 6. From a plot of the corresponding rate-constants with temperature, an activation energy of 20.9 kcal.mole^{-1} for the peeling-off reaction was calculated. This value is almost identical with the activation energy[11] of the peeling-off reaction of amylose (21.2 kcal.mole^{-1}).

So far only the effect of the monovalent bases on polysaccharides have been considered. It is important, however, to consider divalent bases in investigations of this type, since it has been shown that divalent cations have a promotional effect on chain termination of (1→4)-linked polymers[12,34,35] and enhance the rate of peeling in the case of (1→3)-linked carbohydrates[31].

In the present study however, it was found that the rate of degradation of the (1→3)-linked polysaccharide (laminaran) was identical for both sodium and strontium hydroxide at the same concentrations (Fig. 4). This apparent discrepancy is probably due to the low alkali concentration (0.05N) used in Kenner and Richard's experiments[31]. At alkali concentrations below 0.1N there is not sufficient ionization for the monoanion to reach the maximum rate [see Fig. 5 and eqns. (*1*) and (*10*)]. However, by the addition of divalent cations, the small amount of dianion formed may be stabilized[11]. The effective anionic concentration is thereby increased through a shift of the equilibrium of reaction sequence (*1*) to the right. The rate is consequently increased as shown by eqn. (*2*).

A comparison of the rate of degradation of the three (1→3)-β-D-glucans with amylose at 56° in 0.1N sodium hydroxide is shown in Fig. 7. The data are plotted as

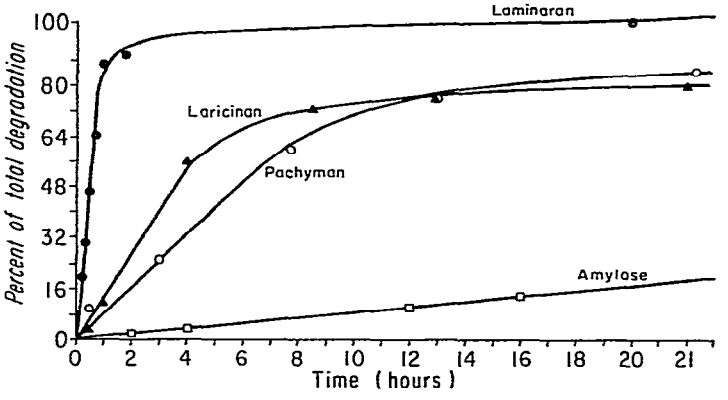

Fig. 7. Comparison of the degradation of (1→3)-β-D-glucans with that of amylose (d.p. ~820), in 0.1M NaOH at 56°.

percent of total degradation. The differences in the rate of degradation of the three (1→3)-β-D-glucans are primarily due to differences in degrees of polymerization (d.p.). For example, laminaran[7], having a d.p. of only 25 shows a higher rate compared to laricinan[36] (having a d.p. of 165), which in turn, is higher than that of pachyman[36] of d.p. 700. To eliminate the effect of d.p., approximate rate-constants for the (1→3)-β-D-glucans calculated from eqn. (*13*) are shown in Table II. The rate constants for laminaran (10.9 h^{-1}) and laricinan (11.5 h^{-1}) at 56° in 0.1M sodium hydroxide are approximately equal. However, pachyman exhibits a much higher rate-constant (29.6 h^{-1}) under these conditions. While no definite conclusions can be based on a difference of this magnitude because of uncertainties in the determination of d.p.,

the higher rate for degradation of pachyman may be real, and derive from the extent and position of branching in the structure of the polymer. For example, Kenner and Richards[37] have shown that substitution of the 6-hydroxyl group with an *O*-methyl group in both 3-*O*-methyl- and 4-*O*-methyl-D-glucose enhances the rate of degradation.

The maximum degradation was independent of alkali concentration, and amounted to 42% for laminaran (in accordance with earlier work[13]), 45% for pachyman, and only 30% for laricinan. Alditol end-groups have not been identified in either pachyman or laricinan, and blockage of reducing end-groups by alditol glycosides is therefore unlikely. Some stable aldonic acid and-groups may be present in the laricinan preparation that was isolated from chlorite holocellulose of larch wood, but similar groups are not likely to be present in pachyman. Consequently, branching is presently the most probable factor causing incomplete degradation of the two polysaccharides.

Branches attached to the 4- and 6-positions of chain units would not be likely to disrupt the degradation of the main chain, but the side chains themselves would obviously remain undegraded in peeling. Branches at the 2-position may behave in the same fashion, judging from the behavior of an analogous compound, 2,3-di-*O*-methyl-D-glucose in lime-water[38]. It was found that, after a normal β-elimination, the 2-methoxyl group remained attached to the resulting α,β-unsaturated derivative.

In fact, Hoffmann and Timell[3] have postulated a branched structure for laricinan, and Timell[36] has found evidence for branches at the 2-positions of pachyman. This hypothesis is further supported by the increased solubility of all of these polymers when compared with the linear (1→3)-β-D-glucan, callose[39,40], which is totally insoluble in all non-degrading solvents except 20% dinitrogen tetraoxide in *N*,*N*-dimethylformamide.

EXPERIMENTAL

Preparations. — Insoluble laminaran from *Laminaria cloustoni* frond was purchased from Pierce Chemical Co., Rockford, Ill. (carbohydrate content[19], 91%; glucose, 97%; mannitol, 2%; fucose[41] less than 2%; $[\alpha]_D^{27}$ −10° in very dilute alkaline solution). Samples of purified pachyman and laricinan were kindly supplied by Professor T. E. Timell, State University of New York College of Forestry.

Kinetic runs. — Distilled water was boiled to remove dissolved oxygen and carbon dioxide prior to the preparation of all solutions. The polysaccharide (5 mg) was dissolved in the appropriate base in a volumetric flask (25 ml). Aliquots (1 ml) of the alkaline polysaccharide solution were transferred to ampoules, thoroughly deaerated with nitrogen gas, sealed, and immersed in a constant temperature bath, as described previously[11]. At intervals, ampoules were removed from the bath, cooled rapidly, and opened. The alkaline polysaccharide solution was neutralized quickly with the appropriate concentration of hydrochloric acid (1 ml). The carbohydrate content of two or three samples (1 ml) of the neutralized solutions was then determined by using the phenol–sulfuric acid reaction[19]. Calibration curves were

prepared by using anhydrous D-glucose in solutions containing the appropriate amount of sodium chloride. The experimental rate-constants were obtained by determining the initial slopes of the polysaccharide-degradation curves. The initial contents of end-groups $[Gr]_0$ were estimated from number-average molecular weight values determined by Professor T. E. Timell.

A kinetic run was also made wherein periodic samples (1 ml) of alkali-degraded laminaran (0.1M NaOH, 56°) were treated with sodium borohydride (50 mg), kept for 12 h, acidified with 1.5M sulfuric acid (1 ml), and analyzed by the phenol–sulfuric acid reaction. No significant degradative differences were noted as a consequence of of this borohydride reduction.

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REFERENCES

1 G. G. ALLAN, P. G. JOHNSON, Y-Z. LAI, AND K. V. SARKANEN, Part II, *Chem. Ind.* (London), (1971) 127; Part I, *Carbohyd. Res.*, 17 (1971) 234.
2 A. E. CLARKE AND B. A. STONE, *Rev. Pure Appl. Chem.*, 13 (1964) 134.
3 G. C. HOFFMANN AND T. E. TIMELL, *Wood Sci. Technol.*, 4 (1970) 159.
4 C. W. FORD AND E. PERCIVAL, *J. Chem. Soc.*, (1965) 7035.
5 A. BEATTIE, E. L. HIRST, AND E. PERCIVAL, *Biochem. J.*, 79 (1961) 531.
6 A. E. CLARKE AND B. A. STONE, *Biochim. Biophys. Acta*, 44 (1960) 161.
7 E. PERCIVAL AND R. H. MCDOWELL, *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, New York, 1967, p. 53.
8 N. NANDA AND H. TOMINAGA, *Marine Biol.*, 2 (1969) 228.
9 S. A. WARSI AND W. J. WHELAN, *Chem. Ind.* (London), (1957) 1573.
10 R. L. WHISTLER AND J. N. BEMILLER, *Advan. Carbohyd. Chem.*, 13 (1958) 289.
11 Y-Z. LAI AND K. V. SARKANEN, *J. Polym. Sci., Part C*, 28 (1969) 15.
12 D. W. HAAS, B. F. HRUTFIORD, AND K. V. SARKANEN, *J. Appl. Polym. Sci.*, 11 (1967) 587.
13 W. M. CORBETT AND J. KENNER, *J. Chem. Soc.*, (1955) 1431.
14 R. M. ROWELL, P. J. SOMERS, S. A. BARKER AND M. STACEY, *Carbohyd. Res.*, 11 (1969) 17.
15 R. M. ROWELL, personal communication.
16 C. H. BAMFORD AND J. R. COLLINS, *Proc. Roy. Soc., Ser. A.*, 204 (1951) 62, 85.
17 W. M. CORBETT AND J. KENNER, *J. Chem. Soc.*, (1954) 3274.
18 D. J. MACLAURIN AND J. W. GREEN, *Can. J. Chem.*, 47 (1969) 3947, 3957.
19 M. DUBOIS, K. A. BILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
20 T. J. PAINTER, *Chem. Ind.* (London), (1963) 36.
21 B. LINDBERG, O. THEANDER, AND J.-E. UDDEGARD, *Svensk Papperstid.*, 69 (1956) 360.
22 J. J. CHRISTENSEN, J. H. RYTTING, AND R. M. IZATT, *J. Chem. Soc., (B)*, (1970) 1646.
23 V. A. DEREVITSKAVA, G. S. SMIRNOVA, AND A. A. ROGOVIN, *Dokl. Akad. Nauk. SSSR*, 141 (1961) 1090.
24 F. URBAN AND P A. SHAFFER, *J. Biol. Chem.*, 94 (1932) 697.

25 J. THAMSEN, *Acta Chem. Scand.*, 6 (1952) 270.
26 J. M. LOS AND L. B. SIMPSON, *Rec. Trav. Chim.*, 76 (1957) 267.
27 H. L. DOPPERT AND A. J. STAVERMAN, *J. Polym. Sci.*, 4 (1966) 2367; 2763.
28 S. A. RYDHOLM, *Pulping Processes*, Interscience Publishers, John Wiley & Sons, Inc., New York, 1965, p. 153.
29 J. U. NEF, *Ann.*, 376 (1910) 1.
30 J. C. SOWDEN, *Advan. Carbohyd. Chem.*, 12 (1957) 35.
31 J. KENNER AND G. N. RICHARDS, *J. Chem. Soc.*, (1957) 3019.
32 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, (1960) 1938.
33 W. M. CORBETT AND J. KENNER, *J. Chem. Soc.*, (1954) 1789.
34 G. MACHELL AND G. N. RICHARDS, *Tappi*, 41 (1958) 12.
35 R. L. COLBRAN AND G. F. DAVIDSON *J. Text. Inst.*, 52 (1961) T73.
36 T. E. TIMELL, personal communication.
37 J. KENNER AND G. N. RICHARDS, *J. Chem. Soc.*, (1956) 2916.
38 J. KENNER AND G. N. RICHARDS, *J. Chem. Soc.*, (1956) 2921.
39 G. O. ASPINALL AND G. KESSLER, *Chem. Ind.* (London), (1967) 1296.
40 G. KESSLER, *Ber. Schweiz. Botan. Ges.*, 68 (1968) 5.
41 E. P. CROWELL AND B. B. BURNETT, *Anal. Chem.*, 39 (1967) 121.