145. *Random Depolymerisations of Polysaccharides in which the Lower Oligosaccharides are Protected from Further Scission.*

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By an extension of Kuhn's statistical treatment of random depolymerisation, it is shown that when a polysaccharide is randomly degraded in such a way that the lower oligosaccharides, once liberated, are protected from further cleavage, the overall yield of these oligosaccharides is much greater than when no protection is provided, and that the largest protected oligomer is obtained in the highest yield.

The results agree with those observed experimentally for degradations of this type, and emphasise the potential value of these degradations in structural polysaccharide chemistry.

ONE of the most valuable methods for investigating polysaccharide structure entails partial depolymerisation by aqueous acid, followed by separation and identification of the fragments. Usually, only those fragments ranging in size from disaccharides to heptasaccharides are suitable for study, as larger ones are difficult to purify and identify unequivocally. A major disadvantage is that the yields of useful oligosaccharides are often low and, therefore, not representative of the original macromolecule.

In an earlier communication,[1] it was suggested that the yield of oligosaccharides could be improved if a non-dialysable catalyst were used for the hydrolysis, and the oligosaccharides were dialysed continuously from the reaction mixture as they were liberated (cf. Hash and King [2]). This was confirmed [3] for hydrolysis of inulin by water-soluble polystyrenesulphonic acid, and the technique has also been applied to enzymic hydrolysis of cellulose [2] and hemicelluloses,[1,4] autohydrolysis of sulphated polysaccharides,[5] and acid-hydrolysis of mucopolysaccharides.[6]

In this paper, some theoretical aspects of this type of degradation are considered, in an attempt to determine the extent to which the yields of the various oligosaccharides should differ from those obtained when no protection is provided for the oligosaccharides. For convenience, the two types of degradation are referred to as "protected" and "unprotected," respectively.

Theory.—In random unprotected degradation of a linear polymer, the yield of an oligomer at any stage of the reaction can be calculated statistically.[7,8] For a degree of scission s, the probability that a particular linkage in the polymer molecule is broken is s, and the probability that it is not broken is $(1 - s)$. Therefore, the probability that a particular fragment containing n monomer units (*i.e.*, an n-mer) is split from within a chain is $s^2(1 - s)^{n-1}$, whereas the probability that it is split from the end of a chain is $s(1 - s)^{n-1}$. Since there are $(N - n - 1)$ ways of choosing an n-mer from within a polymer chain N units in length, and two ways of choosing one from the end of a chain, the total probability of an n-mer existing is given by

$$(N - n - 1)s^2(1 - s)^{n-1} + 2s(1 - s)^{n-1}.$$

The yield Y_n of the n-mer is then given by

$$Y_n = (ns/N)(1 - s)^{n-1}[2 + (N - n - 1)s]. \quad (N > n) \tag{1}$$

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[1] Painter, *Canad. J. Chem.*, 1959, **37**, 497.
[2] Hash and King, *Science*, 1954, **120**, 1033.
[3] Painter, *Chem. and Ind.*, 1960, 1214.
[4] Timell, *Chem. and Ind.*, 1959, 999; Bishop and Perila, *Canad. J. Chem.*, 1961, **39**, 815.
[5] Painter, *Chem. and Ind.*, 1959, 1488.
[6] Painter and Morgan, *Nature*, 1961, **191**, 39.
[7] Kuhn, *Ber.*, 1930, **63**, 1503.
[8] Montroll and Simha, *J. Chem. Phys.*, 1940, **8**, 721.

Equation (1) was originally derived by Montroll and Simha.[8] When N is large compared with n, a close approximation to Y_n is given by Kuhn's equation:[7]

$$Y_n = ns^2(1 - s)^{n-1}. \tag{2}$$

The maximum possible yield of an n-mer in an unprotected degradation can now be calculated. For simplicity, Kuhn's work [7] is considered first. From the first derivative of equation (2),

$$\mathrm{d}Y_n/\mathrm{d}s = 2ns(1 - s)^{n-1} - n(n - 1)s^2(1 - s)^{n-2}; \tag{3}$$

it follows that Y_n reaches a maximum when $s = 2/(n + 1)$, and that the maximum yield is given by

$$Y_n(\text{max.}) = n\left(\frac{2}{n+1}\right)^2\left(\frac{n-1}{n+1}\right)^{n-1}. \tag{4}$$

The maximum yield of n-mer in a protected degradation can also be calculated from equation (3). The positive term in the right-hand side of equation (3) represents the increase in the amount of n-mer due to the breakdown of all fragments larger than n-mer. Therefore the definite integral of this term, $2n\int_0^1 s(1 - s)^{n-1}\cdot\mathrm{d}s$, which simplifies to $2/(n + 1)$, represents that proportion of monomer units which, during the total degradation of polymer to monomer, exist at some stage as discrete molecules of n-mer.[7] However, when the n-mer is protected from further degradation, but no higher oligomers are protected, this integral also represents the total yield of the protected n-mer at the end of the reaction. Hence for a protected degradation,

$$Y_n(\text{max.}) = 2/(n + 1). \tag{5}$$

It is observed that, for all values of n greater than unity, the right-hand side of equation (5) is greater than that of equation (4). Some examples are given in Table 1.

TABLE 1.

n	1	2	3	4	5	6
Equation (4)	1·00	0·298	0·188	0·138	0·110	0·091
Equation (5)	1·00	0·667	0·500	0·400	0·333	0·286
Ratio (5)/(4)	1·00	2·25	2·67	2·89	3·04	3·14

Differentiation of equation (1) shows that, for unprotected random degradation of a finite linear polymer, the value of s corresponding to a maximum yield of n-mer is given by

$$(1 + n^2 + 2n - Nn - N)s^2 + (2N - 4n - 2)s + 2 = 0,$$

where N is the number of monomer units per polymer molecule. The maximum yield is then obtained by substitution for s in equation (1). The results for several values of N and n are shown in Table 2; the figures in parentheses are the optimum values of s. It is observed that the maximum yields of the oligomers from a finite polymer are slightly higher than from an infinite one (Table 1), but that, as N increases, the yields tend rapidly to those given by equation (4).

The final yield of an n-mer in a protected degradation of a finite polymer is obtained, as before, by integration of the positive terms in the first derivative of equation (1). It is found that Y_n(max.) is $2/(n + 1)$, the same as that from an infinite polymer. It follows that, regardless of the molecular size of a linear polymer, the maximum yield of an oligomer in a protected random degradation is always at least twice that obtainable without protection.

TABLE 2.

	Y_n (max.)				
N	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$
3	0·333 (0·500)	—	—	—	—
4	0·316 (0·548)	0·222 (0·333)	—	—	—
5	0·308 (0·577)	0·209 (0·366)	0·169 (0·250)	—	—
6	0·304 (0·596)	0·202 (0·391)	0·159 (0·272)	0·137 (0·200)	—
7	0·302 (0·608)	0·198 (0·408)	0·153 (0·290)	0·129 (0·215)	0·115 (0·167)

This reasoning can now be extended to calculate the yields when more than one oligomer is protected. It is convenient first to consider the example in which both the dimer and the trimer, but no other oligomer, is protected. Since the trimer can be formed from all fragments higher than trimer, the final yield is $2/(n + 1)$, which in this case is one-half. The dimer, however, can be formed only from fragments higher than trimer; therefore the yield is two-thirds $[2/(n + 1)$ where $n = 2]$, less that fraction of monomer units which would have yielded dimer had the trimer not also been protected. The last quantity is two-thirds of the yield of trimer, because equation (5) is valid for the protected degradation of any fragment, regardless of its size. The final reaction product will then consist of trimer (one-half), dimer (one-third), and monomer (one-sixth).

The yields for any homologous series of protected oligomers are calculated in the same way. For example, when the dimer, trimer, and tetramer are the only protected fragments, the yield of the tetramer, $2/(n + 1)$, is two-fifths; the yield of trimer is one-half less one-half of two-fifths, which is three-tenths; and the yield of dimer is two-thirds, less two-thirds of two-fifths, less two-thirds of three-tenths, which is one-fifth. The product will then consist of tetramer (40%), trimer (30%), dimer (20%), and monomer (10%).

In general, when all oligomers from the dimer up to and including the x-mer, but no other oligomers are protected, the combined yield of these oligomers at the end of the reaction is:

$$\frac{2}{x+1} + \sum_{i=1}^{x-2}\left\{\frac{2}{(x-i+1)}\prod_{j=0}^{i-1}\frac{(x-j-1)}{(x-j+1)}\right\}$$

In this expression, the yield of the x-mer is $2/(x + 1)$, and the yield of the $(x - i)$-mer is:

$$\frac{2}{(x-i+1)}\prod_{j=0}^{i-1}\frac{(x-j-1)}{(x-j+1)} \qquad \begin{array}{l} x = 3,\ 4,\ 5,\ \text{etc.} \\ i = 1,\ 2,\ 3,\ \text{etc.} \end{array}$$

These formulæ were used to calculate the combined yields for a number of homologous series of protected oligomers, and the composition of the product in each case (Table 3).

TABLE 3.

	Yield of all oligomers up to and including					
	dimer	trimer	tetramer	pentamer	hexamer	heptamer
Total (%)	66·7	83·3	90·0	93·5	95·3	96·5
Dimer	66·7	33·3	20·0	13·3	9·5	7·2
Trimer..................		50·0	30·0	20·0	14·3	10·7
Tetramer			40·0	26·7	19·1	14·3
Pentamer				33·3	23·8	17·9
Hexamer					28·6	21·4
Heptamer						25·0

For comparison, the maximum possible combined yields of the same homologous series, and the yield of each component, were calculated for unprotected degradation (Table 4). This was done by using equation (2); the optimum value of s for each series was determined graphically.

TABLE 4.

	Yield of all oligomers up to and including					
	dimer	trimer	tetramer	pentamer	hexamer	heptamer
Total (%)	29·8	46·0	56·7	64·1	69·8	73·3
Dimer	29·8	28·5	26·7	25·0	22·9	20·4
Trimer		17·5	18·6	18·8	18·6	17·7
Tetramer			11·4	12·5	13·4	13·7
Pentamer				7·8	9·0	9·9
Hexamer					5·9	6·9
Heptamer						4·7
Optimum s	0·67	0·59	0·54	0·50	0·46	0·42

(None of the figures in these Tables is corrected for the addition of the elements of water to each fragment, as would occur in the hydrolysis of a polysaccharide.)

The foregoing calculations apply only to the random degradation of linear polymers, and take no account of the effect of branching. As a simple example, a polymer containing N monomer units and z single branching points is next considered. The polymer will contain $(z + 2)$ terminal units and $(2z + 1)$ linear chains. If all the linear chains are more than n units in length, the number of ways of choosing an n-mer by the scission of one bond only, that is, an n-mer containing one of the original terminal units, is $(z + 2)$. The number of ways of choosing an n-mer by the scission of two bonds is $N - 1 - n(2z + 1)$, and the number involving the scission of three bonds is $zn(n + 1)/2$. The yield of n-mers for a degree of scission s is therefore given by:

$$Y_n = (ns/N)(1 - s)^{n-1}\left[(z + 2) + (N - 1 - 2nz - n)s + \frac{zn}{2}(n + 1)s^2\right].$$

If N is very large, and the degree of branching (r) is defined as z/N, this equation simplifies to:

$$Y_n = ns(1 - s)^{n-1}\left[r + (1 - 2nr)s + \frac{nr}{2}(n + 1)s^2\right]. \qquad (6)$$

$$(2nr < 1)$$

Application of the calculus to equation (6) as before shows that the final yield of n-mer in a protected degradation, when no higher oligomers are protected, is

$$r + 2(1 - 2nr)/(n + 1) + 3nr/(n + 2).$$

The ratio of this expression to that for a linear polymer, $2/(n + 1)$, is $1 - r(n - 1)/(n + 2)$, and hence the yield from the branched polymer is lower than from a linear one. If, for example, $r = 0·07$, the yield of dimer is lower by 1·75%, and that of heptamer is lower by 4·67%.

For unprotected degradation of the branched polymer, the maximum possible yield of dimer when $r = 0·07$ is 28·6%, and that of heptamer is 6·3%; these figures were obtained graphically, by using equation (6). The corresponding yields from a linear polymer, obtained from equation (4), are 29·8% and 7·8%, respectively.

These calculations show that, even for quite highly branched polymers, the maximum yields of the various oligomers differ very little from those given by equations (4) and (5). For very heavily branched polymers, the equation describing the degradation will involve terms in $s^4(1 - s)^{n-1}$, and terms of still higher order. However, all the terms will contain the factor $(1 - s)^{n-1}$, and the first derivative of the equation, like equation (3), will contain an equal number of positive and negative terms; therefore the final yield of any oligomer in a random protected degradation must always be greater than the maximum yield in an unprotected random degradation.

Discussion.—The Tables show that it is theoretically possible, in a random protected degradation, to obtain a series of oligosaccharides which are small enough to be readily separated by chromatography, and unequivocally identified, in a yield representing well over 90% of the original polysaccharide. This would be impossible in an unprotected degradation. These statements should be generally valid, regardless of the molecular size or degree of branching of the polysaccharide.

In random protected degradation of a linear polymer, the final yields of the protected fragments increase linearly with increasing degree of polymerisation, the highest protected oligomer being obtained in the highest yield (Table 3); the contrast with an unprotected degradation (Table 4) is striking. It is clear that protected degradations should be particularly useful for the isolation of higher oligosaccharides. Table 1 shows that the ratio of the maximum yields of an oligomer, in protected and unprotected degradations, respectively, increases with increasing degree of polymerisation.

In practice, the degradation of a polysaccharide by aqueous acid is never truly random. Even with a simple homopolymer like cellulose, smaller fragments are hydrolysed faster than larger ones,[9] and it can therefore be expected [10] that the yields of the lower oligosaccharides will be smaller than those predicted on the assumption of random cleavage. However, inasmuch as the acid-hydrolysis of many polysaccharides approximates to a random degradation, the foregoing conclusions should be qualitatively correct. A theoretical study of certain types of non-random depolymerisation is in progress.

Another factor which in practice modifies the yields of oligosaccharides calculated above is the impossibility of effecting instantaneous protection of an oligosaccharide. In the method involving continuous dialysis,[1-6] every fragment, from the moment it is liberated in the reaction mixture, is subject to further hydrolysis while diffusing to and through the membrane. Since larger oligomers diffuse more slowly than smaller ones, it is to be expected that, in general, the yields of the oligomers will not increase linearly with degree of polymerisation as shown in Table 3, but will pass through a maximum and then decline. This is in agreement with practical experience.[3] The size of the oligomer obtained in highest yield, and the average molecular weight of the hydrolysate, can be varied simply by varying the rate of dialysis relative to the rate of hydrolysis.[3]

Recent work [11] on the hydrolysis of diethylaminoethyl ethers of starch by polystyrenesulphonic acid suggests that much faster protection of oligosaccharides than that afforded by continuous dialysis is possible, and it is hoped that further work will afford yields of oligosaccharides approaching those shown in Table 3.

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[9] Freudenberg, Kuhn, Dürr, Bolz, and Steinbrunn, *Ber.*, 1930, **63**, 1510; Freudenberg and Blomqvist, *Ber.*, 1935, **68**, 2070.

[10] Meyer and Gonon, *Helv. Chim. Acta*, 1951, **34**, 294.

[11] Painter, *J.*, 1962, 3932.