The Degradation of Carbohydrates by Alkali. Part IX. Cellobiose, Cellobiulose, Cellotetraose, and Laminarin.*

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Cellobiose and cellobiulose suffer degradation by alkali corresponding to that recorded in other instances (*J.*, 1953, 2245; 1954, 1789). Cellotetraose and laminarin undergo peeling processes which are regarded as illustrative of the course of alkaline degradation of hydro- and oxy-celluloses.

THIS series of papers originated from consideration of the behaviour of carbonyl oxycelluloses towards alkali. It was thus necessary to extend our previous studies of disaccharides from this standpoint to the case of cellobiose and of cellobiulose, after preparing the latter from cellobiose by the usual procedure with lime-water. The course of the degradation in each case to D-gluco*iso*saccharinic acids and D-glucose conformed to expectation, the rates of reaction approximating closely to those of lactose (Corbett and Kenner, *J.*, 1953, 2245) and lactulose (*idem*, *J.*, 1954, 1789) respectively, and is to be similarly formulated.

Cellobiose is the ultimate member, apart from glucose itself, of the series of hydrocelluloses. These result from the acidic degradation of cellulose, and so are liable to arise during its separation from the other constituents of wood by the sulphite method. Murumow, Sack, and Tollens (*Ber.*, 1901, **34**, 1427), having prepared such a product by the action of sulphuric acid on cellulose, converted it by treatment with calcium hydroxide

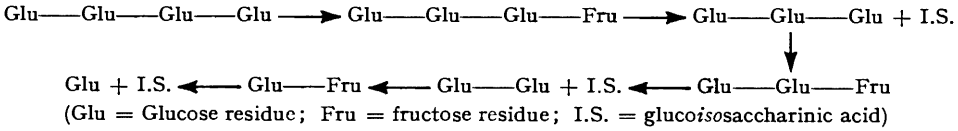
TABLE 1. *Degradation of cellotetraose by lime-water at* 25°.

Time (hr.)	Glucose units decompd. (10^{-5} mole) (1 ml. sample)	Decompn. (%)	Acids produced (eq./mole) (back-titration resin method)		Initial detection of components by paper chromatography
7	0·230	4·7	0·135	0·155	
24	1·037	21·0	0·910	0·880	Cellotriose, cellotriulose, cellobiose, cellobiulose
48	1·912	38·8	1·865	1·680	Glucose
72	2·282	46·3	2·530	2·045	Fructose
168	2·719	55·2	3·520	3·045	
192	2·984	60·5	3·645	2·975	
216	3·007	61·0	3·685	2·960	

into a mixture of essentially D-*iso*saccharinic acids with what was regarded as unchanged cellulose, but it is obviously desirable to supplement this broad picture of the alkaline degradation of mixed hydrocelluloses by a kinetic study in the case of a definite entity belonging to this group of compounds. Table 1 summarises the observations made on a solution in oxygen-free lime-water (25 ml.; 0·0397N) at 25° of cellotetraose (0·2048 g.).

* Part VIII, *J.*, 1954, 3281.

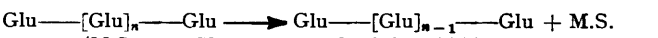
According to the ideas developed in this series, the following scheme should represent the course of the alkaline degradation, and a corresponding one should apply to that of hydrocelluloses in general:



(Glu = Glucose residue; Fru = fructose residue; I.S. = gluco*iso*saccharinic acid)

The relative reactivities of the tetraose, triose, and biose are likely to be approximately equal, and it is therefore not surprising that some glucose arises after decomposition has attained only at most *ca.* 40%.

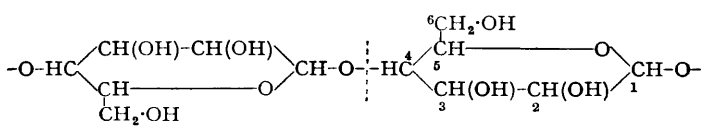
Unfortunately individual macrohydrocelluloses are not at present accessible. The products examined by Tollens and his collaborators were certainly mixtures and, as they stated, contaminated with unchanged cellulose (cf. Schwalbe and Becker, *J. pr. chem.*, 1920, **100**, 19). However, other oligosaccharides with a free carbonyl group and a 1 : 4-, a 1 : 3-, or, less directly (cf. Corbett and Kenner, *J.*, 1954, 3281), a 1 : 6-chain structure should be liable to a similar peeling process. We have, therefore, turned to laminarin with its chain of 1 : 3-linked glucose residues (Connell, Hirst, and Percival, *J.*, 1950, 3494; Percival and Ross, *J.*, 1951, 720). This has the advantage that no rearrangements should be requisite between successive stages of the peeling process, formulated as follows:



(M.S. = D-Glucometasaccharinic acid)(n = *ca.* 40)

In fact, this reaction constitutes a most convenient source of D-glucometasaccharinic acids (B.P. Appln. 21,242/1953) and the general course of the degradation of " insoluble " laminarin was such that, in accordance with this equation, the rate of formation of D-glucometasaccharinic acids corresponded to that of laminarin degradation expressed in terms of glucose equivalents, though the reaction ceased when the degree of degradation amounted to about 48%. This was due to some inhibitive variation in chain structure because reactivity of the residue towards alkali was revived by partial hydrolysis with oxalic acid. As to the nature of the variation, Peat, Whelan, and Lawley (*Biochem. J.*, 1954, **54**, xxxiii) detected gentiobiose and ββ-trehalose among the products of acid hydrolysis of " insoluble " laminarin. The former is indicative of 1 : 6-branching and it is obvious that if the action of alkali should generate a metasaccharinic acid carrying in the 6-position a chain of hexose residues these would not be subject to further degradation. On the other hand a 1 : 1-trehalose linking is irreconcilable with a chain system of 1 : 3-linkages carrying a free aldehydic group at one end as required by our experiments, and so must be part of a separate system. Some indication of such heterogeneity of the laminarin was supported by chromatographic evidence of the transient appearance of glucose and fructose among the products of alkaline degradation—they should only arise when the end of a 1 : 3-chain system is reached. The result of hypoiodite estimation of aldehyde groups (compare Connell, Hirst, and Percival, *loc. cit.*) in our unreactive polysaccharide residues, whilst definitely smaller than in the original material, is far from the negligible value which might have been expected. However, it is not intended to pursue these matters.

It is evident from the following formulation of a portion of a cellulose chain that the presence of a carbonyl group in either the 2- or the 3-position of the right-hand ring will render the chain subject to scission by alkali as indicated owing to formation of a common doubly charged ion:



The left-hand scission product would constitute the anion of a hydrocellulose liable to peeling and, as indicated by the Head's results previously cited (Corbett and Kenner, *J.*, 1953, 2245), should also arise if the carbonyl group is in position 6. The connection

between oxy- and hydro-celluloses detected by Tollens (*Ber.*, 1901, **34**, 1434) is thus explained and is technically important in the viscose process and in the " tendering " of cellulose by oxidation.

Corbett and Kenner (*J.*, 1954, 1789) mentioned that the acids produced during the degradation of lactulose could not be satisfactorily determined by observing the decrease in alkalinity of the solution. With cellobiose, cellobiulose, and cellotetraose we experienced similar difficulty, as illustrated in the Table 1 and have found that it is not due to monosaccharides or to saccharinic acids produced during the degradation. Since it is possible to estimate reasonably accurately the acids produced by removal of all cations by ion-exchange resin, and then titrating the acids thus produced, the apparent excess of acid found by the usual method seems to arise from absorption of calcium hydroxide by the 1 : 4-linked glucosans. No such absorption has been detected in any other type of glucosan and the comparative stability of complexes towards acids indicates that they differ in nature from those occurring in the familiar sucrose–lime case.

Experimental

Cellobiose.—A solution (100 ml.) of chromatographically pure cellobiose (0·8636 g.) in oxygen-free lime-water (0·0425N) was kept at 25°. Aliquot portions were withdrawn periodically and run into excess of washed (3 × 25 ml.) Amberlite IR-120(H) resin (2·3 g.). After filtration and washing of the resin with water (3 × 10 ml.) the solution was titrated against 0·01N-sodium hydroxide (phenolphthalein). This procedure was adopted in preference to treatment of the aliquot sample with sulphuric acid and back-titration after a certain period because the latter, as the following instance illustrates, furnished unexplained higher results: (*a*) back-titration after (1) ¼ hr., 1·318 acid equivs.; (2) ½ hr., 1·315; (3) 1 hr., 1·311; (*b*) resin treatment, 1·185. Mono- and di-saccharides were determined by Corbett's method (*Chem. and Ind.*, 1953, 1285). On this basis, Table 2 summarises the observations.

TABLE 2. *Degradation of cellobiose by lime-water at* 25°.

Time (hr.)	Cellobiose decompd. (%)	Monoses produced (moles/mole)	Saccharinic acids produced [1] (equiv./mole)	Total acids produced (equiv./mole)	Substances found by paper chromatography [2]
1	0·8	0·006	—	0·038	Cellobiulose
2	1·1	0·006	—	0·058	
3	1·9	0·022	—	0·054	Cellobiulose
4	4·6	0·050	—	0·054	
5	5·0	0·061	—	0·086	Glucose
6	6·6	0·083	—	0·123	
7	7·5	0·087		0·123	
24	39·0	0·441	0·380	0·478	Fructose
30	49·5	0·540	0·400	0·595	
48	69·7	0·707	0·631	0·840	
54	72·0	0·702	0·660	0·898	
72	79·7	0·752	0·800	1·038	Cellobiose and cellobiulose (very faint)
79	83·5	0·772	0·785	1·080	
144	90·2	0·756	1·030	1·305	No disaccharide

[1] Cf. Kenner and Richards, *J.*, 1954, 1784. [2] Ketoses detected by naptharesorcinol spray, aldoses and ketoses by silver nitrate spray. Salient features only of the chromatographic observations are noted.

Cellobiulose.—Application to cellobiose of the procedure detailed for lactulose (Corbett and Kenner, *J.*, 1954, 1789) yielded a white highly hygroscopic amorphous *ketose*, $[\alpha]_D^{23}$ (equil.) −60·1° (*c*, 2·40 in H_2O), reducing power (ferricyanide) 1·40 (glucose 1·00) (Found: C, 41·7; H, 7·0. $C_{12}H_{22}O_{11}$ requires C, 42·1; H, 6·5%).

Table 3 summarises observations made on a solution (50 ml.) of 0·4767 g. in oxygen-free lime-water (0·0424N) at 25°. In consequence of the formation of glucose and its gradual degradation to lactic acid from the outset of this experiment, the proportion of alkali used proved insufficient for completion of the degradation, but the results conform to expectation and suffice to demonstrate the nature of the reaction involved.

Cellotetraose.—A solution (25 ml.) of cellotetraose [0·2048 g.; $[\alpha]_D^{23}$ +11·6° (*c*, 1·21 in H_2O);

D.P. 4·18 by hypoiodite], kindly supplied by Dr. D. I. MacGilvray of this Association, in oxygen-free lime-water (0·0397N) was kept at 25°. Duplicate samples (1 ml. each) were withdrawn periodically, one being run on to excess of washed Amberlite IR-120(H) resin and filtered, and the combined filtrate and washings (3 × 10 ml.) titrated against 0·01N-sodium hydroxide (phenolphthalein). The other sample was run into 0·01N-sulphuric acid (5 ml.) and titrated after 15 min. against 0·01N-sodium hydroxide; this solution, acidified with N-sulphuric acid

TABLE 3.* *Degradation of cellobiulose by lime-water at* 25°.

Time (hr.)	Cellobiulose decompd. (%)	Monoses produced (moles/mole)	Saccharinic acids produced (equiv./mole)	Total acids produced (equiv./mole)	Substances found by paper chromatography
1	8·2	0·113	0·125	0·110	Cellobiose, glucose
3	19·1	0·206	0·187	0·242	
5	26·2	0·282	0·258	0·298	
24	65·5	0·565	0·555	0·659	Fructose
48	75·4	0·690	0·660	0·998	
72	76·8	0·594	0·707	Not determined	
144	80·8	0·588	0·746	1·113	

* See notes to Table 2.

(10 ml.), was then heated for 4 hr. under reflux on a boiling-water bath, cooled, exactly neutralised with 0·5N-sodium hydroxide, and diluted to 50 ml.; the glucose content of this solution was estimated, in 2-ml. samples, by the standard Hagedorn–Jensen method (*Biochem. Z.*, **1923**, **135**, 46). Preliminary experiments with both cellotetraose and cellobiose had confirmed the efficacy of this procedure, although in the former case it was necessary to multiply the experimentally determined value by a factor of 1·15. As will be seen from Table 1 the degree of degradation was stable at about 60%, although the solution was alkaline. This may be due to formation of an alkali-resistant complex between residual carbohydrate and calcium hydroxide.

Laminarin.—Samples of " insoluble " and " soluble " material were kindly supplied by the Director of the Institute of Seaweed Research.

A solution of " insoluble " laminarin (Sample No. I.L.7) (50 g.) in an oxygen-free aqueous suspension (1 l.) of calcium hydroxide (50 g.) which had been kept for 8 days at 25° was filtered and treated with the required amount of oxalic acid to remove calcium ions. Concentration of the solution to 500 ml. under reduced pressure at 50° caused separation of polysaccharide (21·4 g.). Evaporation of the filtrate and aqueous washings under reduced pressure at 50° yielded a syrup, successive extractions of which by alcohol (3 × 100 ml.) left further polysaccharide (7·7 g.). The syrup of α- and β-metasaccharins (13·8 g.), remaining after evaporation of the alcoholic extracts at 45°, furnished calcium salts (13·1 g.) from which, when their solution in hot water (31 ml.) was cooled, calcium β-metasaccharinate (5·7 g.) separated as cubic crystals, $[\alpha]_D^{20}$ −23·4° (*c*, 2·05 in H_2O) {Nef, *Annalen*, **1910**, **376**, 95, gave $[\alpha]_D^{20}$ −23·25° (*c*, 4 in H_2O)}; β-metasaccharinolactone, m. p. 87—91° from ethyl acetate $[\alpha]_D^{21}$ +8·27° (*c*, 1·21 in H_2O) (Found: C, 44·1; H, 6·2. Calc. for $C_6H_{10}O_5$: C, 44·4; H, 6·2%), was obtained quantitatively from it (Nef, *loc. cit.*, gave m. p. 92°, $[\alpha]_D^{20}$ +8·2° in H_2O). Gradual addition of alcohol to the mother-liquors caused separation of solid as follows: (1) 0·13 g., $[\alpha]_D^{20}$ −23·4° (*c*, 1·97 in H_2O), from 45% ethanol; (2) 3·13 g., $[\alpha]_D^{21}$ −9·5° (*c*, 2·00 in H_2O), from 54% ethanol; (3) calcium α-metasaccharinate (0·60 g.), $[\alpha]_D^{21}$ −5·0 (*c*, 1·99 in H_2O), from 64% ethanol (Kenner and Richards, *J.*, 1954, 278, found $[\alpha]_D^{21}$ −5·2°); (4) 0·13 g., $[\alpha]_D^{20}$ −8·7° (*c*, 2·07 in H_2O), from 74% ethanol; (5) 0·11 g., $[\alpha]_D^{21}$ −12·3° (*c*, 0·81 in H_2O), on evaporation of the mother-liquors and digestion of the residue with ethanol. Evaporation of the alcoholic liquors yielded 0·27 g., $[\alpha]_D^{22}$ −7·4° (*c*, 2·16 in H_2O). After a solution of polysaccharide (50 g.) (recovered as above) in N-oxalic acid (1·5 l.) had been heated at 100° for 2 hr., excess of calcium hydroxide was added and heating continued for 2½ hr. in a stream of nitrogen. Treatment of the product as above yielded polysaccharide (10·9 g.), calcium β-metasaccharinate (7·9 g.), $[\alpha]_D^{23}$ −22·1° (*c*, 2·0 in H_2O), and crude calcium α-metasaccharinate (11·6 g.), $[\alpha]_D^{23}$ −6·1° (*c*, 2·0 in H_2O).

The following Table records the behaviour at 25° under oxygen-free conditions of a solution of " insoluble " laminarin (No. I.L 4) (0·5750 g.) in warm water (5 ml.) diluted with lime-water to 100 ml. (final normality 0·0398). The acids were determined by back-titration; the glucose produced by hydrolysis of a sample (10 ml.; initially 0·165 mg. of laminarin) with 4N-sulphuric acid (5 ml.) for 1 hr. at 100° was estimated by the Hagedorn–Jensen method. Glucose and fructose were detected after 72 hr., but their concentrations appeared to diminish after 7 days.

Time (hr.)	0·125	1	2	6	23	47
Equiv. of acids produced per glucose unit	0·000	0·014	0·023	0·086	0·240	0·321
Glucose units decompd. (%)	0·0	*	*	11·0	30·6	37·7
Time (hr.)	71	143	215	240	311	
Equiv. of acids produced per glucose unit	0·350	0·386	0·417	0·434	0·424	
Glucose units decompd. (%)	*	*	*	48·5	47·7	

* Not determined.

Similarly, a solution of " soluble " laminarin (50 g.) in an oxygen-free aqueous suspension (1 l.) of calcium hydroxide (50 g.) at 100° furnished after 3 hr. polysaccharide (26·0 g.), and crude mixed metasaccharins (12·3 g.; 10·9 g. after recovery from their filtered acetone solution). From the calcium salts (11·2 g.), prepared and fractionated as described above, were separated, *inter alia*, the β-metasaccharinate (2·4 g.), $[\alpha]_D^{24}$ −23·2° (*c*, 1·17 in H_2O), and the α-salt (4·6 g.), $[\alpha]_D^{24}$ −6·8 (*c*, 1·18 in H_2O). The brucine salt, m. p. 145—148° (Found: N, 5·1. Calc. for $C_{29}H_{38}O_{10}N_2$: N, 4·9%), from the latter showed $[\alpha]_D^{22}$ −24·8° (*c*, 1·13 in H_2O); Nef (*loc. cit.*) gave m. p. 145—150°, $[\alpha]_D^{20}$ −23·1°. Recovered polysaccharide (20 g.), treated as was that from " insoluble " laminarin, yielded polysaccharide (4·8 g.), impure calcium β-metasaccharinate (2·9 g.), $[\alpha]_D^{24}$ −15·6° (*c*, 5·1 in H_2O), and crude calcium α-metasaccharinate (4·2 g.), $[\alpha]_D^{22}$ −6·2 (*c*, 2·1 in H_2O).

No substantial change in the above results ensued on preliminary dialysis of the laminarin against distilled water, followed by treatment with charcoal. Thus after 4 hr. at 100°, in oxygen-free lime-water (0·0415N), the degree of degradation, as measured by acid formation, was constant and amounted respectively for untreated and for treated samples to 39·9 and 33·8% for " insoluble " (I.L.7) and to 19·1 and 19·0% for " soluble " (S.L.5) material.

Hypoiodite chain-length determinations on the samples of laminarin employed above gave values of 42 (I.L.4), 38 (I.L.7), and 72 (S.L.5) respectively, whilst those of the corresponding unreactive materials derived from them were 166, 194, and 114 respectively.

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