906. *The Alkaline Degradation of Polysaccharides. Part II.* The Alkali-stable Residue from the Action of Sodium Hydroxide on Cellulose.*

By G. Machell and G. N. Richards.

The alkali-stable polysaccharide resulting from the treatment of cotton hydrocellulose with hot dilute, oxygen-free sodium hydroxide has been partially hydrolysed and D-gluco*meta*saccharinic acids isolated and identified. α-D-Glucosaccharinic acid was tentatively identified by paper chromatography. It is concluded that these acids originate by alkaline rearrangement of the reducing end-group of the cellulose molecule and so impart stability to alkali.

Degradation of cellulose by boiling dilute sodium hydroxide ultimately yields a polysaccharide which is stable to oxygen-free alkali,[1] and in view of the extensive losses of α-cellulose which occur, for example, in the hot alkali refining of wood-pulps [2] there is technological interest in the nature of the alkali-stable material. Richtzenhain *et al.*[1] have shown that the extent of degradation taking place before the attainment of alkali-stability is related to the degree of polymerisation of the original cellulose, and consequently to the number of reducing end-groups at which the degradation is known to commence. Further, there is evidence [3] that alkali stability is induced by conversion, during the degradation, of a normal reducing glucose end-group into a carboxylic acid, and two suggestions have been made as to the nature of this acid, although no attempt has been made to identify it. Richtzenhain and Abrahamsson [4] pointed out that a reducing end-group might undergo rearrangement to a saccharinic acid, while still remaining attached to the cellulose molecule, whereas Lindberg [5] was of the opinion that the alkali-stability may be due to oxidation of the reducing end-groups to carboxyl groups, either by traces of oxygen or by a " crossed " Cannizzaro reaction between the end-group and a soluble carbonyl degradation product. The latter type of reaction might also produce sorbitol as the end-unit of the chain, and this would also result in alkali-stability. However, Richtzenhain *et al.*[1] have also suggested that the ultimate alkali-stability may have a physical rather than a chemical cause.

The aim of the present work was to isolate and identify the alkali-stable end-group or groups. Alkali-stable hydrocellulose was prepared by degrading cotton hydrocellulose in 0·5N-sodium hydroxide at 100° in the absence of oxygen, conditions comparable to those of Richtzenhain *et al.*,[1] and the degradation was accompanied by a slight increase in the weight-average degree of polymerisation (D.P.). This increase, although occurring in a reaction which involves progressive shortening of cellulose molecules and a weight loss of more than 50%, is not unexpected. The system may be related to the acidic hydrolysis of hydrocellulose; here the D.P. falls only very slightly over a considerable range of weight loss, and Sharples [6] has shown that the effective hydrolysis occurs only at the ends of the cellulose micelles, causing a progressive shortening analogous to that postulated for the alkaline degradation. The resultant lack of effect on the weight-average D.P. depends on the molecular-weight distribution in hydrocellulose.[6] The observed increase in D.P. during alkaline degradation may therefore result from a combination of this effect with a preferential alkaline extraction of material of low D.P.

* Part I, preceding paper.

[1] Cf., *e.g.*, Richtzenhain, Lindgren, Abrahamsson, and Holmberg, *Svensk Papperstidn.*, 1954, **57**, 363.
[2] Cf., *e.g.*, Richter, *TAPPI*, 1955, **38**, 129.
[3] Samuelson and Wennerblom, *Svensk Papperstidn.*, 1954, **57**, 827; Clibbens, Geake, and Ridge, *J. Textile Inst.*, 1927, **18**, T277.
[4] Richtzenhain and Abrahamsson, *Svensk Papperstidn.*, 1954, **57**, 538.
[5] Lindberg, *ibid.*, 1956, **59**, 531.
[6] Sharples, *Trans. Faraday Soc.*, 1957, **53**, 1003.

The presence of carboxylic acid groups in the product was demonstrated by its ability to absorb methylene-blue, but the relation of this absorption to an absolute carboxyl content is in doubt, particularly in the present instance where the acidic groups must be concentrated at the extremities of the cellulose micelles and so possibly not wholly accessible to a stoicheiometric interaction with the methylene-blue.

The initial step in the isolation of the group responsible for alkali-stability was acidic hydrolysis of the alkali-stable hydrocellulose, and Sharples's observations [6] on the acidic hydrolysis of hydrocellulose suggested that hydrolysis by a dilute, non-swelling mineral acid would preferentially dissolve end-units from the micelles. Further, Rogovin *et al.*[7] have shown that cellobiose and cellobionic acid are hydrolysed at similar rates, from which it follows that the presence of an aldonic acid type of grouping would not materially affect the rate of hydrolysis of the neighbouring glycosidic link. Probably therefore comparatively mild hydrolysis of the alkali-stable hydrocellulose would remove sufficient of the acidic end-grouping to permit its isolation. Accordingly, alkali-stable hydrocellulose was hydrolysed in N-hydrochloric acid at 100° for varying times, and the fall in methylene-blue absorption determined. The results are shown in Table 1. It was observed that the

TABLE 1. *Acidic hydrolysis of alkali-stable hydrocellulose.*

	Methylene-blue absorption (mmoles/100 g.)			Methylene-blue absorption (mmoles/100 g.)	
Hydrolysis (hr.)	Initial	After NaOH treatment	Hydrolysis (hr.)	Initial	After NaOH treatment
0	3·9	—	4	1·8	2·3
1	2·4	3·4	7	1·7	2·2
2	2·0	2·9	17	1·3	1·3

methylene-blue absorption of the hydrolysed alkali-stable hydrocellulose increased on treatment with 0·1N-sodium hydroxide in the cold, which does not affect hydrocellulose itself. This is unlikely to be due to hydrolysis of lactones since the latter would probably be decomposed in the methylene-blue solution, which is buffered at pH 8.[8] It is possible, however, that this effect may indicate esterification of the acidic end-group by glucose in the hydrolysis medium, followed by release of the polysaccharide acid on treatment with alkali.

In accordance with the observation of Richtzenhain *et al.*,[1] when alkali-stable hydrocellulose, from which most of the alkali-stable groups had been removed by a more stringent hydrolysis, was again treated with alkali at 100°, it suffered the same extent of degradation as the original hydrocellulose.

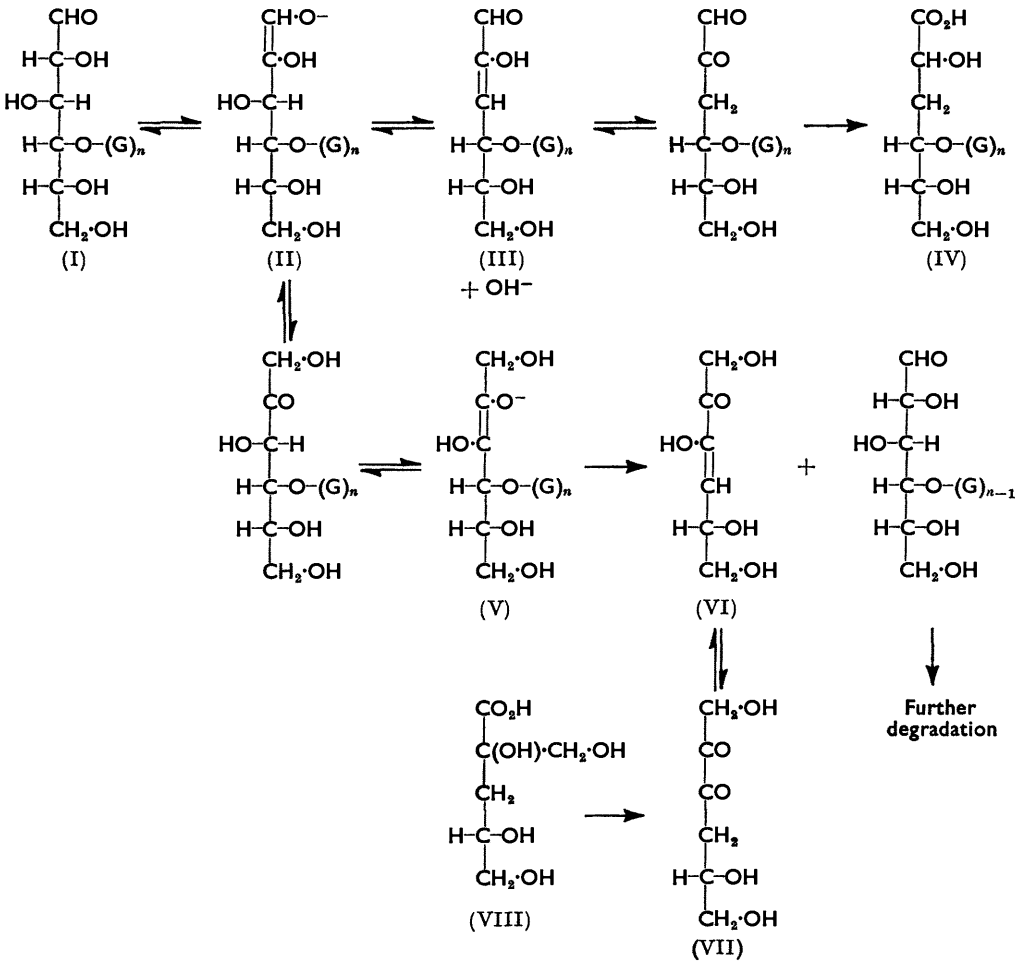
A large-scale hydrolysis of alkali-stable hydrocellulose yielded an acidic solution containing the stable end-group with a much larger amount of glucose. Hydrochloric acid was conveniently removed from this solution by treatment with methyldi-*n*-octylamine,[9] and the remaining acids separated from glucose by sorption on a suitable anion-exchange resin.[10] Examination of the neutral solution by paper chromatography, after enzymic destruction of glucose, revealed no neutral products other than cellobiose and traces of 5-hydroxymethylfurfuraldehyde, the latter presumably arising by acidic degradation of glucose. Elution of the acidic products from the resin, and examination by paper chromatography, indicated that the main constituents were α- and β-D-gluco*meta*saccharinic acid. There were traces of other acids, one of which corresponded on the paper chromatogram to α-D-glucosaccharinic acid. Pure α- and β-D-gluco*meta*saccharinic acid were isolated in the form of their lactones by preparative paper chromatography and identified as

[7] Rogovin, Konkin, and Rymashevskaya, *Khim. i Fiz. Khim. Vysokomolecul. Soedinenii Doklady 7-oi Konf. Vysokomolecul. Soedineniyam*, 1952, 140; *Chem. Abs.*, 1954, **48**, 4449.
[8] Cf. Davidson and Nevell, *J. Textile Inst.*, in the press.
[9] Smith and Page, *J. Soc. Chem. Ind.*, 1948, **67**, 48.
[10] Machell, *J.*, 1957, 3389.

alkaloid salts. Hydrolysis of the original hydrocellulose in this way did not afford any acidic products, but did give the neutral compounds mentioned above.

It is concluded that during the degradation of cellulose by oxygen-free alkali, two types of competing reaction occur, which may be referred to as " degradation " and " stopping " reactions. Degradation reactions have been discussed earlier (Part I), and of these the most important are those leading to D-gluco*iso*saccharinic acid (VIII). The scheme indicates the predominant " degradation " and " stopping " reactions in terms of recent theories [11] of saccharinic acid formation.

The " stopping " reaction (I ⟶ IV) occurs at a much slower overall rate than the degradation (I ⟶ VIII), chiefly owing to the mass-law effect in reactions (II) ⟶ (III), and (V) ⟶ (VI).[12] Thus there is considerable degradation of a given cellulose molecule before the " stopping " reaction renders it stable.

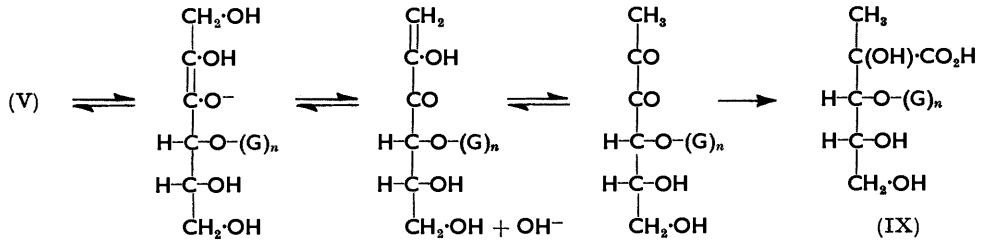


(I) = cellulose molecule of D.P. (n + 1). (IV) = alkali-stable residue.

The tentative identification of traces of α-D-glucosaccharinic acid [IX; H in place of $(G)_n$] in the hydrolysis products from the alkali-stable residue is in accord with the fact that the same saccharinic acid is a major product of the alkaline degradation of glucose.

[11] Kenner, *Chem. and Ind.*, 1955, 727, and references therein.
[12] Kenner and Richards, *J.*, 1957, 3019.

This sequence of reactions, however, is presumably less effective in the present instance than that resulting in formation of the *meta*-isomer (I ⟶ IV) owing to the fact that it involves a reactive intermediate (V) which can more readily break down by an alternate route (to VIII).



It is true that the D-gluco*meta*saccharinic acid end-group could have originated alternatively at a branch-chain of any length which was attached to the 3-position of a glucose unit in the main cellulose chain (compare, *e.g.*, the alkaline degradation of laminarin [13]), if this type of branch unit or chain were either present in the original cellulose or produced by acid reversion during preparation of the hydrocellulose.[14] While this cannot at present be disproved, it is regarded, on the evidence available at present, as unlikely to have an important effect on the " stopping reaction."

Experimental

The following solvents and sprays were used for paper chromatography with Whatman No. 1 paper at 25°: solvents *a*, butanol–pyridine–water (6 : 2 : 3); *b*, ethyl acetate–acetic acid–water [15] (10 : 1·3 : 1); *c*, butanol–ethanol–acetic acid–water (45 : 5 : 1 : 49); *d*, phenol–water (4 : 1). Sprays were: *a*, silver nitrate–sodium hydroxide; [16] *b*, hydroxylamine–ferric chloride; [17] *c*, B.D.H. 4·5 indicator; [18] *d*, buffered methyl-red.[19]

Degree of polymerisation (D.P.) of cellulose samples was determined by measurement of the viscosity of solutions of the nitrates in butyl acetate,[20] and methylene-blue absorption by Davidson's method.[21]

Acidity of solutions containing lactonisable acids was determined by adding a 2—4-fold excess of alkali and titrating the whole with acid to pH 9 after 30 min. at room temperature.

Preparation of Hydrocellulose.—American cotton, in the form of card sliver, was dewaxed by extraction (Soxhlet) with chloroform, followed by 95% ethanol, each for 18 hr. Initially, the acidic hydrolysis of the cotton to hydrocellulose was carried out with 2N-sulphuric acid for 6 hr. at 100°, but this gave material which probably contained ·O·SO_3H groups and was therefore abandoned.

Dewaxed cotton (100 g.) was then hydrolysed with 2N-hydrochloric acid (3 l.) for 5½ hr. at 100°, and then in a similar batch of fresh acid for a further 30 min. to hydrolyse any reversion products from the hydrocellulose. The product (80 g.) had a methylene-blue absorption of 0·38 mmoles/100 g., which accords with the results of other workers.[21] The hydrolysis was attended by a decrease in D.P. of the cellulose, from 4600 to 150. This hydrocellulose was used in all subsequent work.

For comparison, a portion of the original cotton was " scoured " by 0·5N-sodium hydroxide at 100° for 24 hr. in the absence of oxygen. The product was washed free from alkali, and then hydrolysed with 2N-hydrochloric acid at 100° for 6 hr. to give a hydrocellulose of methylene-blue absorption 0·22 mmole/100 g.

[13] Corbett and Kenner, *J.*, 1955, 1431.
[14] Sharples, unpublished work.
[15] Moilanen and Richtzenhain, *Acta Chem. Scand.*, 1954, **8**, 704.
[16] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[17] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[18] Nair and Muthe, *Naturwiss.*, 1956, **43**, 106.
[19] S. N. Parikh, J. M. Parikh, and Godbole, *Current Sci.*, 1954, **23**, 53.
[20] Harland, *J. Textile Inst.*, 1955, **46**, T483, and earlier references.
[21] Davidson, *ibid.*, 1948, **39**, T65.

Alkaline Degradation of Hydrocellulose.—Hydrocellulose (50 g.) was dried to constant wt. over phosphoric oxide at 0·1 mm., then suspended in water (750 ml.) through which oxygen-free nitrogen was passed. Oxygen-free N-sodium hydroxide (750 ml.) was added and the mixture heated at 100° in an atmosphere of nitrogen for 20 hr. After external cooling with ice, the residue was filtered off through sintered glass (porosity 4), washed successively with water, 1% acetic acid, water, 95% ethanol, and ether, and dried to constant wt. as above. The yield of alkali-stable residue was 21 g. (42%), methylene-blue absorption 4·2 mmoles/100 g., D.P. 180.

Acid-hydrolysis of Alkali-stable Hydrocellulose.—A number of samples of alkali-stable hydrocellulose (2 g.) were hydrolysed in N-hydrochloric acid (100 ml.) at 100° for varying times, and the methylene-blue absorption of the residues determined. These residues were then shaken with 0·1N-sodium hydroxide at 20° for 1 hr.; their methylene-blue absorptions were barely increased. Results are given in Table 1.

Alkaline Degradation of Hydrolysed Alkali-stable Hydrocellulose.—Alkali-stable hydrocellulose (5 g.) was hydrolysed with 2N-hydrochloric acid (250 ml.) at 100° for 18 hr., leaving a residue (4·1 g.) of methylene-blue absorption 1·0 mmole/100 g. A portion (3·2 g.) of this was degraded in 0·5N-sodium hydroxide at 100° for 20 hr. in the absence of oxygen, and the alkali-stable residue (1·47 g., 46%) recovered as before.

Hydrolysis of Alkali-stable Hydrocellulose and Isolation of the Soluble Products.—Alkali-stable hydrocellulose (50 g.), prepared as indicated above, was dialysed for several days to ensure complete removal of water-soluble acidic degradation products. The material had a yellow colour, which was completely removed by extraction (Soxhlet) with ethanol for 24 hr. The ultraviolet absorption spectrum of the extract had a peak 2700 Å. Concentration of the extract produced a gelatinous precipitate on which the chromatophore appeared to be adsorbed, and attempts to extract it from this residue by ether were unsuccessful. The extracted alkali-stable hydrocellulose was hydrolysed in N-hydrochloric acid (500 ml.) at 100° for 2 hr., and the residue separated and washed with water (1 l.) at 70°. After decolorisation with charcoal and cooling, about 98% of the mineral acid was removed from the combined filtrate and washings by Smith and Page's method,[9] *i.e.*, shaking the aqueous solution with methyl di-*n*-octylamine (128 g.) in chloroform (1400 ml.) and separating the layers. The aqueous layer was evaporated to about 200 ml. and slowly run through a column (0·8 cm. diam.) packed with washed Amberlite resin IR-120(H) (20 ml.) to remove cations. The effluent contained chloride which was precipitated by silver carbonate. After filtration, silver ions in solution were removed by passage through Amberlite resin IR-120(H) (20 ml.). A slightly acidic (pH 4) solution resulted, and a small portion of the solution was concentrated for paper chromatography.

Solvent *a* with spray *a*, and solvent *b* with spray *b*, revealed much glucose and a smaller amount of an acid lactone. The latter gave a diffuse spot of R_F *ca.* 0·35, and there was extensive " streaking " due to overloading of the glucose.

The main bulk of the solution was then separated into neutral and acidic fractions by using De-Acidite FF (micro-bead, 2% cross-linked) in the carbonate form.[10] This resin (5 g.) was stirred with the concentrated hydrolysate (50 ml.) for 24 hr. and the resin then transferred to a narrow column and washed with water (500 ml.). The filtrate and the initial washings (200 ml.) containing the neutral products were retained (see below).

After passage of " AnalaR " N-ammonium carbonate (100 ml.) through the resin, elution of acid was complete. The excess of ammonium carbonate in the effluent was decomposed by evaporation under reduced pressure, and the diluted residue (50 ml.) of ammonium salts passed through washed Amberlite resin IR-120(H) (10 ml.) to regenerate the free acids. Back-titration after treatment with excess of alkali, as above, indicated 0·44 meq. of acid.

In the same way, hydrolysis of the original hydrocellulose (50 g.) gave a neutral (see below) and an acidic fraction. The latter contained 0·13 meq. of acid and gave positive tests for hydrochloric and sulphuric acid, which arose from the reagents and ion-exchange resins. Paper chromatography of the acidic solution established the absence of the organic acids detected below. Therefore, by subtraction of this figure from the amount of acid isolated from the alkali-stable hydrocellulose, the true yield of acid peculiar to the latter should be 0·31 meq.

Identification of the Acid obtained by Hydrolysis of Alkali-stable Hydrocellulose.—Paper chromatography of the acid obtained as above, by using solvent *b* and spray *b*, revealed two main lactone spots of roughly equal intensity (R_F 0·33 and 0·38). In addition, there were traces of lactones with R_F 0·46, 0·53, and 0·70. β- and α-D-Gluco*meta*saccharinolactone and α-D-saccharinolactone had R_F 0·32, 0·37, and 0·52 respectively. Application of spray *a* to a

second chromatogram established the absence of glucose and the presence of small amounts of materials of R_F 0·10 and 0·21: only the former gave an acid reaction on the chromatogram with spray *c*. Conclusive identification of these products, in particular the two main lactones, depended on their prior separation, which was effected by large-scale paper chromatography.

To establish the feasibility of this approach, and to assess the efficiency of recovery, an attempt was made to separate in this way the authentic α- and β-D-gluco*meta*saccharinolactone. A solution of comparable amounts of these lactones with some free acids was obtained by treating a solution of the appropriate calcium salts with excess of Amberlite IR-120(H) resin. The lactone–acid mixture (1·60 meq., 0·259 g. calc. as lactone) was then transferred to a single sheet 22 × 24 in. of Whatman No. 3 MM. paper, which had been previously irrigated with the developing solvent for 2 days, and dried. After development in solvent *b* for 16 hr. the positions of the two lactones were determined by the application of spray *b* to indicator strips: the two lactones were almost separated. The horizontal strips bearing the two lactones, a 1 cm. wide mixed fraction between them, and the section containing the slower-moving free acids, were separately eluted with water. Paper chromatography of the two lactone fractions as above showed that both lactones were free from contamination with the anomer. Titration of aliquot portions of the eluted fractions as described above indicated a recovery of 47% in the combined lactone fractions, and a further 13% in the acidic eluate.

The acidic solution isolated from alkali-stable hydrocellulose was then transferred to No. 3 MM. paper and separated as above. The acidic material of R_F 0·10 was re-chromatographed in solvent *b*, and with spray *b* gave lactone spots corresponding to α- and β-D-gluco*meta*saccharinolactones as well as the spot due to the free acids (spray *c*). The material of R_F 0·21 was divided into two parts which were heated with N-hydrochloric acid and N-sodium hydroxide severally at 100° for 1 hr. Recovery of the organic products, followed by chromatography in solvent *b*, showed that these treatments were without effect on the material. This product was not investigated further.

Paper chromatography of the two main lactone fractions (A and B) from the above separation showed them to be pure, and their R_F values were compared with those of a number of authentic lactones, with solvents *b* and *c* and spray *b*. Details are shown in Table 3.

TABLE 3.

	Solvent *b*		Solvent *c*	
	R_F	R_S *	R_F	R_S *
D-Glucono-δ-lactone	0·27	0·53	0·32	0·63
β-D-Gluco*meta*saccharinolactone	0·32	0·62	0·32 †	0·64
Lactone A	0·32	0·61	0·32	0·64
Lactone B	0·36	0·70	0·38	0·74
α-D-Gluco*meta*saccharinolactone	0·37	0·72	0·38 †	0·75
α-D-Gluco*iso*saccharinolactone	0·39	0·76	0·43	0·85
α-D-Glucosaccharinolactone	0·52	1·0	0·51	1·0

* S = α-D-glucosaccharinolactone.
† In an earlier communication [22] these values were transposed in error.

Evaporation of the aqueous solutions of lactones A and B gave dried residues of 37 mg. and 34 mg. respectively. These proved to be contaminated with hemicellulose extracted from the chromatography paper. There was insufficient of each lactone present to permit exact determination by titration, but, on the basis of the results of previous experiments, the amounts of the α- and β-forms were probably of the order of 15 mg. and 20 mg. respectively.

β-D-*Gluco*meta*saccharinolactone*.—The residue (A) was extracted with acetone–ether (1 : 4), the solvent removed, and the residue taken up in ethyl acetate. The syrup obtained on evaporation did not crystallise: it was heated in water (5 ml.) with excess of strychnine at 100° for 4 hr. After cooling, the unchanged alkaloid was extracted with chloroform (4 × 10 ml.), and the solution evaporated to dryness at 40°/20 mm. The residue crystallised from absolute ethanol, then sintering and decomposing at 180—185° (Nef [23] gives decomp. 180—190° for strychnine β-D-gluco*meta*saccharinate).

α-D-*Gluco*meta*saccharinolactone*.—The lactone (B) was freed from hemicellulose as above, but did not crystallise. One portion was converted as above into the strychnine salt, m. p.

[22] Kenner and Richards, *J.*, 1954, 1784.
[23] Nef, *Annalen*, 1910, **376**, 1.

145—148° (Nef [23] gives m. p. 145—147° for strychnine α-D-gluco*meta*saccharinate). The brucine salt had m. p. 143—145°, alone or mixed with brucine α-D-gluco*meta*saccharinate.

Neutral Products from the Hydrolysis of Hydrocellulose and Alkali-stable Hydrocellulose.—In each case the aqueous solution of the neutral products (1·7 g. from alkali-stable hydrocellulose) obtained as described above was diluted to 250 ml. An aliquot portion (50 ml.) of each solution was diluted to 200 ml. and incubated with a glucose dehydrogenase preparation ("DeeO," kindly supplied by Takamine Laboratory, Inc., New Jersey, U.S.A.) (*ca.* 10 mg.) for 18 hr. at 35° in a stream of oxygen. In both cases, complete oxidation of the glucose to gluconic acid was achieved, and the acid was removed completely by stirring the concentrated solution (50 ml.) with De-Acidite FF (micro-bead, 2% cross-linked) (5 g.) in the carbonate form. Paper chromatography of the residual solutions, with solvent *d*, established the absence of glucose (R_F 0·41), and the presence of cellobiose, R_F 0·34, and traces of a second component, R_F 0·92, in both cases. The ultraviolet spectrum of both solutions showed max. at 2850 Å, with min. at 2450 Å, which are characteristic of 5-hydroxymethylfurfuraldehyde.[24] Application of spray *d* failed to detect any sorbitol (R_F 0·51) in either solution. In a separate experiment, it was shown that the glucose dehydrogenase had no measurable effect on an aqueous solution of sorbitol.

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[24] Wolfrom, Schuetz, and Cavalieri, *J. Amer. Chem. Soc.*, 1948, **70**, 514; Love, *Biochem. J.*, 1953, **55**, 126.