905. *The Alkaline Degradation of Polysaccharides. Part I. Soluble Products of the Action of Sodium Hydroxide on Cellulose.*

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The major soluble products of the degradation of cotton hydrocellulose by boiling, oxygen-free 0·5N-sodium hydroxide have been identified and in several cases their relative amounts have been determined. D-Gluco*iso*-saccharinic and formic acid are produced in greatest amount under the conditions used.

Much of the earlier structural work on polysaccharides, particularly the starches and hemicelluloses, was carried out on materials which had been subject to vigorous alkaline treatments. Recent work on the alkaline degradation of *O*-substituted hexose derivatives [1] indicated, however, that this type of treatment, particularly of a polysaccharide containing 1 : 3-, 1 : 4-, or 1 : 6-glycosidic links, is likely to result in stepwise degradation from the reducing end of the molecule. Nevertheless the generalisations derived from the work on *O*-substituted hexoses suggest possible application in the determination of polysaccharide structure by alkaline degradation. For example the nature of the saccharinic acid product is dependent on the position of substitution, *i.e.*, the position of the polysaccharide linkage, and the effect of branch points on alkaline degradation should be specific and to some extent predictable. These generalisations, however, were mostly derived from experiments in lime-water at room temperature, which were made in order to simplify the reaction systems involved, and little is yet known of the effects of other alkalis and the higher temperatures which have often been used in alkaline treatments of polysaccharides.

For work on polysaccharides, cotton cellulose was the obvious choice since its structure and reactivity have been much studied. Further, it combines chemical and stereochemical purity with ready availability. Alkaline degradation of cellulose in the hot alkaline refining of wood pulps for the rayon industry causes considerable losses of α-cellulose, but no extensive systematic study has yet been made of the soluble products of this reaction system, and much of the work on wood pulps has been complicated by degradation of hemicelluloses.

Davidson's original suggestion [2] that attack by alkali occurs only at the reducing end of the cellulose molecule has received much further experimental support. Thus it has been shown that modification of the reducing end-group by oxidation,[3a] reduction,[3b] or glycosidation [3c] can render cellulose stable to alkali, and it is known [4] that D-gluco*iso*-saccharinic acid is a major product. Theories of saccharinic acid formation [5] are in accord with this, and more particularly it has been shown [6] that cellotetraose in lime-water undergoes the expected stepwise degradation from the reducing end, which leads to *iso*saccharinic acid. However, D-gluco*meta*saccharinic,[4c] formic, acetic, lactic, and glycollic [7] acid are also produced by alkaline degradation of cellulosic materials, and it seemed probable that other types of alkaline degradation occurred simultaneously, affording a complex mixture of products.

The aim of the present work was to identify and determine the relative amounts of the most important products of the degradation of cellulose by hot, dilute, aqueous sodium

[1] Kenner and Richards, *J.*, 1957, 3019, and earlier references.
[2] Davidson, *J. Textile Inst.*, 1934, **25**, T174.
[3] Meller, *TAPPI*, (*a*) 1952, **35**, 72; (*b*) 1953, **36**, 366; (*c*) Reeves, Schwartz, and Giddens, *J. Amer. Chem. Soc.*, 1946, **68**, 1383.
[4] (*a*) Murumow, Sack, and Tollens, *Ber.*, 1901, **34**, 1427; (*b*) Richtzenhain and Abrahamsson, *Svensk Papperstidn.*, 1954, **57**, 538; (*c*) Green, *TAPPI*, 1956, **39**, 472.
[5] (*a*) Isbell, *J. Res. Nat. Bur. Stand.*, 1944, **32**, 45; (*b*) Kenner, *Chem. and Ind.*, 1955, 727, and references therein.
[6] Corbett and Kenner, *J.*, 1955, 1431.
[7] Chesley, Montgomery, and Sandborn, U.S.P. 2,750,414.

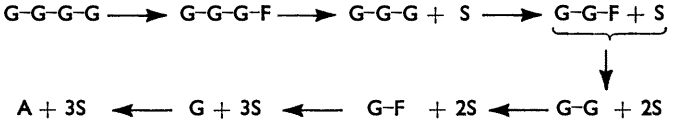
hydroxide (conditions corresponding approximately to those of rayon pulp-refining). Cotton hydrocellulose was chosen as substrate because its method of preparation and high crystallinity gave maximum probability of normal end-groups to each molecule, and chemical uniformity along the chains. In order to reduce possible secondary rearrangements of primary products of the degradation, reactions were restricted to relatively short times, and oxygen was excluded in order to avoid possible autoxidation of reactive intermediates. Thus hydrocellulose was boiled with oxygen-free 0·5N-sodium hydroxide for 30 min., and the soluble products were isolated and identified as indicated in the Experimental section, the results being summarised in Table 1. The rapid formation of γ-lactones in presence of a sulphonic acid-substituted resin, and subsequent partial separation of lactonisable from non-lactonisable acids, is noteworthy.

TABLE 1. *Soluble acids from treatment of hydrocellulose with* 0·5N-*sodium hydroxide at* 100°.

Acid	R_L value (solvent *a*)	Identification [a]	Yield (% carbon return)
Unidentified acid *	0·34	P.C.	
Unidentified lactone	0·43	P.C.	
β-D-Gluco*meta*saccharinic	0·52 (lactone)	P.C.	
αβ-D-Gluco*iso*saccharinic	0·58 (lactone) 0·19 (acid)	C.	*ca.* 44
βγ-Dihydroxybutyric *	0·60	P.C.	
C_5 *meta*-Saccharinic	0·69 (lactone)	P.C.	
αβ-Dihydroxy-α-methylpropionic	0·70	P.C.	
Glycollic	0·74	C.	
Unidentified acid	0·80	P.C.	
α-D-Glucosaccharinic ‡	0·82 (lactone)	P.C.	
Lactic	1·00	C.	4·3
Dihydroxybutyric	1·02 (lactone)	P.C.	
Lactyl-lactic	1·11	P.C.	
Formic	(Volatile)	C.	7—8
Acetic	(Volatile)	T.	<2

* Reacts rapidly with spray *b*. † May conceal traces of α-D-gluco*meta*saccharinic acid.
‡ May also include C_5 *meta*saccharinolactone.
[a] P.C. = paper chromatography; C = crystalline derivative; T = qualitative test.

Many of the minor products, particularly lactic acid, are known to be produced in considerable amount by the alkaline degradation of glucose under these conditions, and in this respect it is particularly important to realise the effect of complete degradation of a given cellulose molecule. It follows from Corbett and Kenner's results [6] that this would liberate the non-reducing end-unit of the molecule as glucose:



(G = glucose; F = fructose; S = *iso*saccharinic acid; A = acids from alkaline degradation of glucose.)

This will be more important if the soluble oligosaccharides either extracted or produced during the degradation are degraded more rapidly than the insoluble cellulose. The glucose liberated would undergo degradation and rearrangement which, under the prevailing conditions, would be expected [8] to yield mainly lactic acid. To investigate the importance of this effect the hydrocellulose was separated into fractions of high and low degree of polymerisation (D.P.) by the method commonly used for α-cellulose determination, and the relevant fractions are therefore referred to as α- and β-hydrocellulose respectively. Both fractions contained only the cellulose II crystalline structure and it is assumed that any contribution from degradation of the non-reducing end-group would be emphasised in the composition of the products of degradation of the β-fraction. The

[8] (*a*) Shaffer and Friedemann, *J. Biol. Chem.*, 1930, **86**, 345; (*b*) Evans, *Chem. Rev.*, 1942, **31**, 537.

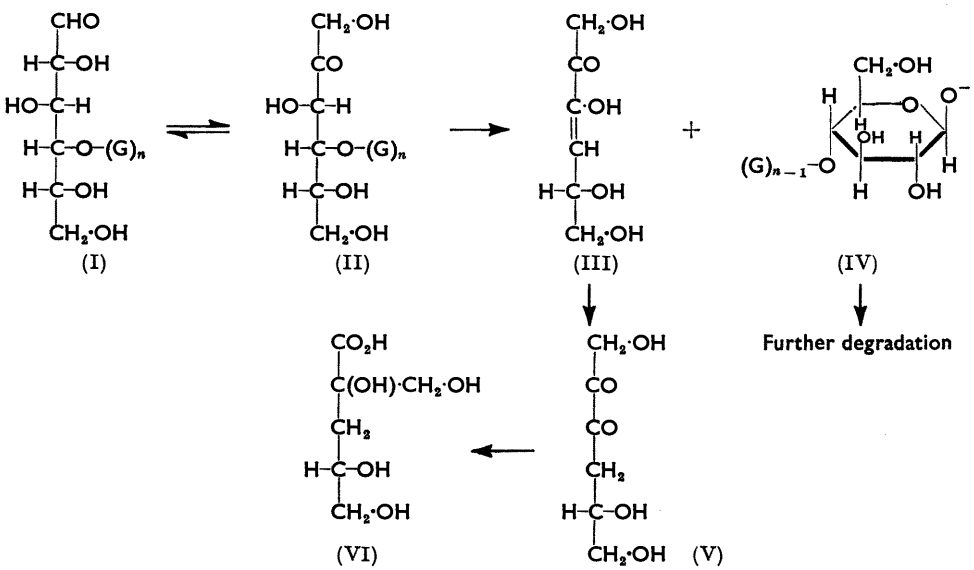
production of formic and lactic acid on alkaline degradation of these fractions is recorded in Table 2.

TABLE 2. *Degradation of α- and β-hydrocellulose by 0·5N-sodium hydroxide.*

	α-Hydrocellulose			β-Hydrocellulose		
Time (min.)	Total acid (meq./g.)	Formic acid (%)	Lactic acid (%)	Total acid (meq./g.)	Formic acid (%)	Lactic acid (%)
5	0·15	27	6·0	0·8	27	5·7
10	—	—	—	1·1	—	5·3
15	0·3	28	6·2	1·4	—	5·7
30	0·5	29	6·0	2·0	35	5·4
240	0·9	34	6·8	—	—	—

Discussion.—The present results leave no doubt that D-gluco*iso*saccharinic acid (VI) is the major product of the degradation of cellulose by hot dilute sodium hydroxide.

Infrared analysis of the non-volatile acids, and periodate oxidation of the lactonisable acids, confirmed this, and quantitative paper chromatography indicated a carbon return of approximately 44% as this acid under the prevailing conditions. The sequence of reactions leading to formation of this acid and resulting in progressive stepwise degradation of the cellulose molecule (I) from the reducing end-group has been discussed by Corbett and Kenner.[6]

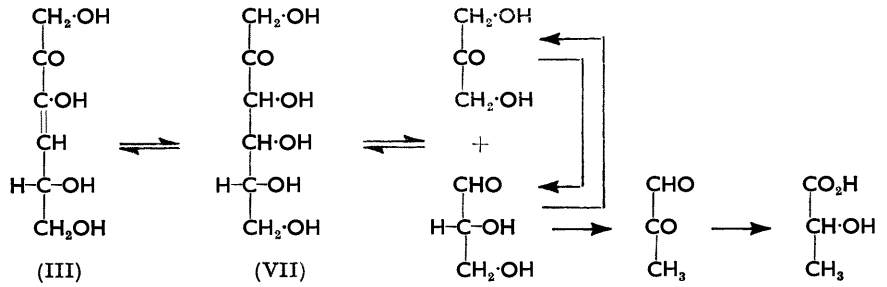


For the first time, however, it is now evident that other products are formed to the extent of approximately 56% carbon return in the degradation of pure cellulose by oxygen-free sodium hydroxide. The results in Table 2 show that degradation of the non-reducing end-group does not contribute to the formation of this mixture of acids, and the observed lactic acid could not arise by a reversed aldol condensation of the fructosyl intermediate (II) (cf. ref. 9) since ionisation of the 4-hydroxyl group is prevented by substitution. In the case of the keto-enol (III), however, it is reasonable to suppose that a slow, competing addition of hydroxyl anion might occur, leading to formation of a ketohexose mixture (VII), alkaline degradation of which would yield a mixture containing mostly lactic acid, together with most of the acids in Table 1.

The observed relative yields of lactic acid are much lower than those recorded by Chesley *et al.*[7] for similar degradations at 240°, although the yields of acetic and formic acid were similar in both systems. This indicates that several types of reaction, having very

[9] Kenner and Richards, *J.*, 1954, 1784, and references therein.

different activation energies, may be involved. It is probable that there are additional sources of most of the other products, the most likely being degradation and rearrangement of the diketone (V), fragmentation of the reducing end-group (I), or an alternative degradation of the substituted glucosyl anion (IV).



Formic acid is evidently a particularly important product and its yield both in relation to those of total acids and of acetic acid is quite different from that obtained by similar alkaline degradation of glucose.[8b] Certain possibilities regarding its formation can, however, be eliminated. There is no indication of the formation of appreciable amounts of free formaldehyde during the reaction—it was not detected, for example, in the volatile neutral products. Also the complete absence of methanol from the products eliminates the possibility that formic acid arose by a Cannizzaro reaction between two molecules of formaldehyde; and similarly a crossed Cannizzaro reaction between formaldehyde and another monocarbonyl compound would be expected to yield a stable product in sufficient amount to permit its detection. The possibility that small amounts of unremoved atmospheric oxygen were causing oxidation of the reducing end-grouping to formic acid and a substituted arabonic acid is eliminated by the results of the succeeding paper.

Experimental

The following solvents and sprays were used for paper chromatography on Whatman No. 1 paper at 25°: solvents *a*,[10] ethyl acetate–acetic acid–water (10 : 1·3 : 1); *b*, ethanol–aq. ammonia (*d* 0·88)–water (8·5 : 1·4 : 1); *c*,[11] butan-1-ol–ethanol–water (4 : 1·1 : 1·9). Sprays *a*,[12] hydroxylamine–ferric chloride; *b*,[13] sodium metaperiodate–potassium permanganate; *c*,[14] B.D.H. "4·5" indicator; *d*,[15] bromophenol-blue–citric acid; *e*,[16] 1% solution of diphenylamine in acetic acid–concentrated sulphuric acid (39 : 1 freshly prepared); *f*,[17] silver nitrate–sodium hydroxide.

Analytical Methods.—(a) *Formic acid.* 0·1N-Formic acid (50 ml.) was distilled to dryness under reduced pressure from a bath at 60°, a splash-head being used to avoid spray contamination, and the distillate collected in a receiver at 0°. Water (5 ml.) was added to the residue and the whole again evaporated to dryness, and this was repeated twice more. Titration of the distillate and residue indicated that all but 2·5% of the formic acid distilled under these conditions. Similar treatment of a lactic acid solution yielded 10% of the acid in the distillate.

Volatile acids from hydrocellulose degradations were boiled under reflux for 2½—3 hr. with an excess of red mercuric oxide. Experiments with authentic samples indicated that this procedure completely removed formic acid, while having no effect on acetic acid, which could be titrated in the resulting solution after filtration. The similar procedure described by Evans and Hass,[18] in which the solution was boiled for only 30 min., resulted in complete destruction of formic acid only when freshly prepared yellow mercuric oxide was used.

(b) *Lactic acid.* Hullin and Noble's procedure [19] was applied with the omission of the lime

[10] Richtzenhain and Moilanen, *Acta Chem. Scand.*, 1954, **8**, 704.
[11] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[12] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[13] Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.
[14] Nair and Muthe, *Naturwiss.*, 1956, **43**, 106.
[15] Kennedy and Barker, *Analyt. Chem.*, 1951, **23**, 1033.
[16] Cf. Dische, *Microchemie*, 1930, **8**, 4.
[17] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[18] Evans and Hass, *J. Amer. Chem. Soc.*, 1926, **48**, 2708.
[19] Hullin and Noble, *Biochem. J.*, 1953, **55**, 289.

treatment. Addition of known amounts of lactic acid to the products of alkaline degradation of hydrocellulose and subsequent determination by this method gave the expected increases, so it was assumed that other products did not interfere. The procedure gave no colour with relevant amounts of authentic α-D-gluco*iso*saccharinic and glycollic acid, and with formic acid gave only a small optical density at 5650 Å.

(c) *Glycollic acid.* Calkins's procedure,[20] when applied to the products of alkaline degradation of hydrocellulose, yielded a solution with maximum absorption at 4650 Å, compared with 5300 Å for authentic glycollic acid. Although this procedure with relevant amounts of authentic α-D-gluco*iso*saccharinic, formic, and lactic acid did not lead to colour formation, addition of known amounts of glycollic acid to the degradation products and subsequent treatment by this method indicated that it was not suitable for the determination of glycollic acid in this case.

Preparation of Cotton Limit Hydrocellulose.—Chemically untreated Sudan-sakel cotton in the form of carded sliver (kindly supplied by Dr. G. F. Davidson of the Shirley Institute) was cut into short lengths, boiled vigorously with 1% sodium hydroxide solution (40 ml./g.) for 8 hr., filtered, and washed with water. The scoured cotton was then boiled with stirring with 2N-hydrochloric acid (40 ml./g.) for 3 hr., the solution decanted from the cellulose powder, an equal volume of fresh acid added, and boiling continued for a further 3 hr. The final product was filtered off, washed with water until neutral and then with ethanol and ether, and dried (P_2O_5) as a fine, white powder.

Fractionation of Hydrocellulose.—Cotton hydrocellulose (80 g.) was stirred gently under nitrogen at room temperature for 1 hr. with 17·5% sodium hydroxide solution (500 ml.) and then diluted by the slow addition of water (375 ml.). After being kept at 0° overnight the solid α-hydrocellulose was separated by centrifuging, washed with water, dilute hydrochloric acid, and water again, and finally with acetone and ether and dried at 0·1 mm. (P_2O_5) as a white powder (25 g.; D.P. 291 by viscosity of cuprammonium solution; *X*-ray diffraction indicated only cellulose II lattice present).

A further sample (80 g.) of hydrocellulose was treated with 17·5% aqueous sodium hydroxide for 1 hr. as above, diluted by the slow addition of water (4·5 l.), and kept at 0° overnight. The liquor was separated by centrifuging and adjusted to pH 5 by the slow addition of concentrated hydrochloric acid with vigorous stirring. The resultant gelatinous precipitate was separated next morning, dialysed, washed with acetone and ether, and dried as above (yield 20 g.). β-Hydrocellulose was a light, pale yellow powder of D.P. 135 (cuprammonium), and *X*-ray diffraction indicated only the cellulose II lattice.

Alkaline Degradation of Hydrocellulose: Qualitative Analysis.—(a) *Volatile products.* An aqueous suspension of hydrocellulose (48·2 g. in 700 ml.) was boiled in a stream of nitrogen with stirring under reflux for 15 min. Oxygen-free 7·5N-sodium hydroxide (50 ml.) was then added and boiling and stirring continued for a further 30 min. in an atmosphere of nitrogen. The resulting suspension was cooled under nitrogen, then filtered, and the solid washed with water (500 ml.), followed by ethanol and ether, and dried (P_2O_5) (yield 43·1 g.). The filtrate and aqueous washings were combined, stirred with freshly washed Amberlite resin IR-120(H) (200 ml.) for 10 min., then passed through a column of the same resin (200 ml.). The acidic effluent and subsequent washings were combined (1990 ml.), an aliquot portion was treated with an equal volume of 0·1N-sodium hydroxide (4-fold excess) at room temperature for 30 min., and then titrated with 0·1N-hydrochloric acid to phenolphthalein. The total acid yield thus determined was 42·8 meq.

The remaining acidic solution (1970 ml.) was boiled and stirred under reflux with excess of calcium carbonate for 10 min., then cooled, filtered, and evaporated to dryness under reduced pressure (yield 6·14 g.); some insoluble black resin (*ca.* 0·1 g.) which separated during the evaporation was rejected. The first portion (50 ml.) of the distillate was collected at −20° and gave a negative test for methanol.[21] To the remainder of the distillate was added concentrated hydrochloric acid (350 ml.), followed by a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid (350 ml.). After 24 hr. at room temperature the resultant orange-coloured precipitate (0·32 g.) was separated (filtrate A) and recrystallised from anisole and then nitrobenzene as fine brown needles, m. p. 245—246° (decomp.) (Found: C, 45·0; H, 3·2; N, 21·5%). After a further 10 days at room temperature the filtrate (A) had deposited a further precipitate (0·24 g.) (m. p. 280°) which, when recrystallised from nitrobenzene as an orange powder, had

[20] Calkins, *Analyt. Chem.*, 1943, **15**, 762.
[21] Denigès, *Compt. rend.*, 1910, **150**, 832.

m. p. 301° (decomp.) alone or in admixture with the pyruvaldehyde derivative (Found: C, 42·1; H, 2·9; N, 25·5. Calc. for $C_{15}H_{12}O_8N_8$: C, 41·7; H, 2·8; N, 25·9%).

The syrupy calcium salts were next dissolved in water and passed through a column of Amberlite resin IR-120(H) (50 ml.). The total acidity of the resulting solution, determined by back-titration of excess of alkali as above, corresponded to 40·0 meq. after completion of sampling. This solution was evaporated to dryness as described above for the determination of formic acid; the total acidity of the distillate by direct titration with sodium hydroxide then corresponded to 14·5 meq., of which 12·5 meq. was formic acid.

Determination of formic acid in an aliquot portion of a solution of the residue from distillation, by boiling with mercuric oxide as above, indicated the presence of a further 0·6 meq. A portion of the distillate was next boiled under reflux for 3 hr. with excess of mercuric oxide, filtered, neutralised with calcium carbonate and evaporated to dryness. The residue gave qualitative tests for acetic and lactic acid. A further portion of the distillate was neutralised with sodium hydroxide, concentrated, and converted into 4-bromophenacyl formate in the usual way. Recrystallised from aqueous ethanol this had m. p. and mixed m. p. 133—134°.

(b) *Preliminary examination of non-volatile acids and identification of* α-D-*gluco*isosaccharinic *acid.* Part of the residue (1·0 g.) from the foregoing distillation was boiled with water (10 ml.) containing excess of calcium hydroxide for 5 min., cooled, and filtered. The filtrate was saturated with carbon dioxide, boiled for 5 min., again cooled, and filtered. The resulting solution was decolorised with charcoal, concentrated to *ca.* 5 ml., and kept at 0° overnight. A small amount (0·06 g.) of calcium α-D-gluco*iso*saccharinate separated and was converted by treatment with Amberlite resin IR-120(H) into the crystalline lactone, m. p. and mixed m. p. 91·5—92·5° after one recrystallisation from ethyl acetate–light petroleum.

Calcium salts were precipitated from the mother-liquors of the above crystallisation by gradual addition of ethanol but, apart from a concentration of the D-gluco*iso*saccharinates in the earlier fractions, no effective fractionation was observed The fractions were treated with Amberlite resin IR-120(H) and examined by paper chromatography in solvent *a* with sprays *a*, *b*, and *c*. The acids which were tentatively identified by this method and by similar chromatography in subsequent experiments are indicated in Table 1 with their R_L values (L = lactic acid): D-gluco*iso*saccharinic acids were clearly the major products, while the minor products were all apparently present in comparable amount. Authentic reference compounds were normally chromatographed in presence of an excess of D-gluco*iso*saccharinolactone, since this often affected the R_L values.

(c) *Identification of lactic acid.* A sample of the total non-volatile acids (7·20 g.), obtained as described above, was shaken at room temperature for 10 min. each with three portions (each 20 ml.) of dry ether. The combined extracts were evaporated to dryness, to yield a viscous liquid (0·20 g.) which by paper chromatography in solvent *a* (sprays *a*, *b*, *c*) contained components corresponding to lactic and lactyl-lactic acid, a dihydroxybutyrolactone, and αβ-D-gluco*iso*saccharinolactone. This syrup was then extracted by shaking for 10 min. at room temperature with three portions (2·5 ml. each) of 9 : 1 ether–*n*-hexane. The extract contained almost pure lactic and lactyl-lactic acid (0·05 g.) which readily gave 4-bromophenacyl lactate, m. p. and mixed m. p. 112—113°.

(d) *Identification of glycollic acid.* Part of the residue (4·30 g.) from the ether-extraction in (c) was further dried at 50°/0·01 mm. over phosphoric oxide for 1 hr. and then dissolved in water (250 ml.). Direct titration to pH 7 showed the presence of 8·4 meq. of free acid, while back-titration of excess of alkali as in (a) indicated 25·7 meq. of total acid. The remaining solution (245 ml.) was stirred at room temperature for 15 min. with Amberlite resin IRA-400 (carbonate form; 25 ml.) and then passed through the same sample of resin in a column during 15 min. The column was subsequently washed with water (25 ml.), the total effluent combined, excess of aqueous ammonia added, and the whole evaporated to dryness. The residue (B) was shown by paper chromatography in solvent *b* (sprays *b*, *d*) to contain mainly D-gluco*iso*-saccharinic acids (R_L *ca.* 0·58). Glycollic (R_L *ca.* 0·72) and lactic acid (R_L 1·00) were also detected and possibly resisted removal by the resin owing to cross- or self-esterification.

The resin carbonate was eluted with N-ammonium carbonate (200 ml.) during 2 hr. and the eluate and washings were evaporated to dryness. A solution of the residue in water (20 ml.) was stirred for 15 min. with Amberlite resin IR-120(H) (20 ml.), then filtered, and the resin washed with water. The filtrate and washings were concentrated to 10 ml., and back-titration after treatment of an aliquot portion with excess of alkali indicated the presence of 0·85 meq. of

acid per ml. Part of this solution (7·5 ml.) was transferred uniformly to two sheets of Whatman No. 3 mm. paper (56 × 61 cm.) and eluted with solvent *a* for 7 hr. at 25°. Indicator strips (causing 6% loss) were treated on alternate sides with sprays *a* and *c*, and the band corresponding to glycollic acid eluted with water. A band intermediate between the glycollic acid and the *iso*saccharinolactone zone contained mixed acids and was rejected. The eluate contained 0·70 meq. of acid (by direct titration with sodium hydroxide), and readily yielded 4-bromophenacyl glycollate, m. p. and mixed m. p. 138—140° after recrystallisation from benzene. Paper chromatography of the eluate indicated mainly glycollic acid with only traces of the supposed α-D-glucosaccharinolactone.

Elution of the relevant band yielded 0·38 meq. of chromatographically pure lactic acid, but lactyl-lactic acid was lost by elution from the bottom of the paper during chromatography. Elution of a band from the origin to a position of R_L 0·4 yielded 0·76 meq. of acid by back-titration of excess of alkali, and this material, when examined as ammonium salts by paper chromatography in solvent *b*, was found to contain D-gluco*iso*saccharinolactone (R_L *ca.* 0·58), and glycollic (R_L *ca.* 0·76), and lactic acids (R_L 1·00), presumably arising by alkaline hydrolysis of " cross-esters."

(e) *Identification of* D-*gluco*iso*saccharinic acids.* A solution (250 ml.) of the ammonium salt residue (B) from the previous experiment was stirred with Amberlite resin IR-120(H) (40 ml.) at room temperature, while at intervals aliquot portions were titrated with 0·01N-sodium hydroxide (phenolphthalein, transient end-point). Total acids were determined by back-titration and lactonisation calculated as follows: 20% (0·5 hr.); 31% (1·5 hr.); 39% (3·0 hr.); 44% (4·5 hr.); 56% (21 hr.); 56·5% (26·5 hr.). After 27 hr. the solution was filtered and stirred for 1 hr. with Amberlite resin IRA-400 (carbonate) (40 ml.) (pH 4·3), then filtered again and shown by back-titration of excess of alkali to contain 7·1 meq. (0·0220 meq./ml.) of lactonised acid (losses by sampling) (solution L).

Part of the solution (300 ml.) was stirred with excess of calcium hydroxide at 60° for 30 min., then filtered, saturated with carbon dioxide, and concentrated to 50 ml. After a further filtration and subsequent concentration to 5 ml. the solution was kept at 0° overnight; calcium α-D-gluco*iso*saccharinate (0·30 g.) then crystallised and was converted in almost theoretical yield into the crystalline lactone, m. p. and mixed m. p. 90—92° (from ethyl acetate–light petroleum). The mother-liquors containing calcium salts were evaporated to dryness (0·90 g.) and crystallisation of the residue from a small amount of water yielded a further amount of the above α-isomer (0·24 g.). The remaining mother-liquors were treated with Amberlite resin IR-120(H) and then brucine in the usual manner, to yield the brucine salts, which crystallised readily from 95% ethanol. The product (0·32 g.) when recrystallised from the same solvent showed m. p. 152—153° alone or in admixture with authentic brucine α-D-gluco*iso*saccharinate (Found: C, 60·2; H, 6·8; N, 4·9. Calc. for $C_{29}H_{38}O_{10}N_2$: C, 60·6; H, 6·7; N, 4·9%).

Fractional precipitation by ether of the mother-liquors from crystallisation of the brucine salts yielded small amounts of later fractions which, when recrystallised several times from ethanol and ether, had m. p. 185—195°, not depressed by admixture with authentic brucine β-D-gluco*iso*saccharinate (Found: N, 5·2%).

(f) *Periodate oxidation of the lactonisable acids.* Part of the lactone solution (L) (25 ml.) from the previous experiment was treated with N-sodium hydroxide (1 ml.) at 60° for 5 min., cooled, and neutralised to pH 9 with hydrochloric acid. 0·2M-Sodium metaperiodate (20 ml.) was next added, and the solution diluted to 50 ml. and kept at room temperature. At intervals aliquot portions (5 ml.) were added to 10% sulphuric acid (10 ml.) containing potasium iodide (1 g.) and titrated with 0·1N-sodium thiosulphate. Periodate was consumed as follows: 2·9 (0·5 hr.); 4·1 (3·0 hr.); 4·4 (6·0 hr.); 4·8 (24 hr.) moles/equiv.

Similar treatment of authentic α-D-gluco*iso*saccharinolactone gave the following results: 2·8 (0·5 hr.); 3·8 (1·5 hr.); 4·3 (3·0 hr.); 4·7 (6·0 hr.); 4·9 (24 hr.) moles/equiv.

(g) *Non-volatile neutral products.* Hydrocellulose (50 g.) was treated with boiling 0·5N-sodium hydroxide (150 ml.) for 15 min. as in (a) above (acid yield 57·2 meq.; recovery of cellulose 43·6 g.). The acidic solution (1 l.) resulting from subsequent treatment with Amberlite resin IR-120(H) was stirred with Amberlite resin IRA-400 (carbonate form, 150 ml.) for 2 hr., then filtered, and the filtrate was stirred overnight with De-acidite FF (carbonate form, micro-bead, 2% cross-linked) (25 ml.). The conductivity of the solution was then 10^{-5} mho and after filtration evaporation gave a colourless syrup (0·16 g.).

Paper chromatography in solvent *c* indicated the following components: (i) corresponding

to glucose (R_F 0·075) (eliminated by treatment of the mixture with glucose dehydrogenase); (ii) corresponding to fructose (R_F 0·08); (iii) reacting as 2-deoxy-D-ribose (R_F 0·34) with spray *e* (blue coloration on heating); (iv) reacting with spray *f* (R_F 0·39), main component; (v), (vi), (vii), reacting with spray *f* (R_F 0·45, 0·54, 0·59); (viii), (ix), reacting on paper with silver nitrate in acetone (R_F 0·70, 0·80).

Elution of a small amount of fractions (viii) and (ix) with water from a paper chromatogram and examination of ultraviolet absorption of the resulting solutions gave absorption maxima as follows: (viii) 2600 Å, shifting to 2950 Å in alkali; (ix) 2450—2500 Å, shifting to 3100 Å in alkali.

Alkaline Degradation of Hydrocellulose: Quantitative Analysis.—(a) *Formic and lactic acid from α- and β-hydrocellulose.* In a typical experiment the hydrocellulose sample (0·5—1·5 g. of α-fraction; 0·25 g. of β-fraction) was heated with oxygen-free 0·5N-sodium hydroxide (10 ml. per g. of α-fraction; 20 ml. per g. of β-fraction) in a stoppered glass tube immersed in a boiling-water bath for the appropriate time. The mixture was then cooled, freshly washed Amberlite resin IR-120(H) (1 ml. per ml. of alkali) added, the whole shaken for 15 min., then filtered, and the resin washed until the filtrate totalled 100 ml. The total acidity of the solution was determined by back-titration of excess of alkali as above, and after suitable dilution of an aliquot portion lactic acid was determined as above. A further aliquot portion of the solution (75 ml.) was distilled and formic acid determined as described above. The results are expressed in Table 2. Probably owing to the heating conditions and absence of agitation the reproducibility of the total acid yield, relative to the amount of hydrocellulose, was *ca.* ±4%. The formic acid yields do not include undistilled formic acid (see above).

(b) D-*Glucoisosaccharinic acid.* A sample of the non-volatile acids obtained by alkali degradation of hydrocellulose as described above was dissolved in water, and the solution shown, by back-titration of excess of alkali, to contain 1·16 meq. of acid per ml. An aliquot portion (1·5 ml.) of this solution was transferred uniformly to a sheet of Whatman No. 3 mm. paper (56 × 61 cm.) and eluted with solvent *a* for 12 hr. at 20°. Suitable indicator strips were treated with sprays *a* and *c* on alternate sides of the paper, and the bands corresponding to α,β-D-gluco*iso*saccharinic acid and its lactone were eluted with water (25 ml., 50 ml. respectively). The acid in each solution was determined by back-titration of excess of alkali and corresponded to 0·09 (acid band) and 0·58 (lactone band) meq. The solution obtained by elution of the lactone band gave a positive test for glycollic acid and contained 0·10 meq. of free acid, determined by direct titration with alkali. Since an aqueous solution of authentic α-D-gluco-*iso*saccharinolactone contained no appreciable amount of free acid after storage at room temperature for 1 day, it is assumed that this free acidity is due to other acids. Besides the lactone, traces of free acids were detected by rechromatographing the concentrated eluates in solvents *a* and *b*.

The above chromatographic separation was repeated with a solution prepared by treatment of calcium α-D-gluco*iso*saccharinate with Amberlite resin IR-120(H) in water. 1·215 Meq. of the acid were added to the paper, of which 0·07 meq. was recovered in the acidic band and 0·77 meq. (0·02 meq. as free acid) in the lactone band, corresponding to a total recovery of 69%. Application of the appropriate factors to the recovery of D-gluco*iso*saccharinic acid from the previous experiment indicates that the maximum yield of this acid corresponds to 55% of the total non-volatile acid equivalents, or 48% after allowance for free acids as above. These figures were in reasonable agreement with a semi-quantitative infrared absorption analysis of a sample of the non-volatile acids, comparison being with an amorphous sample of α,β-D-gluco-*iso*saccharinolactone which had been purified by chromatography on carbon–Celite. The analysis was carried out on an absorption band thought to be connected with the lactone ring system, and the proportion of lactone in the mixture was determined by direct and back-titration in the usual manner.

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