105. *The Alkaline Degradation of Glucose and of Some of its Acetyl Derivatives.*

By W. M. Corbett and A. Margaret Liddle.

The alkaline degradation of glucose has been studied in detail. In solutions of hydroxyl-ion concentration 0·01—0·05 g.-ions per l., the yield of total acid is inversely related to the hydroxyl-ion concentration, being between 2·5 and 1·5 equiv. The increase in acidity is mainly due to an increase in yield of formic acid. It has also been shown that gluco-metasaccharinic is the main saccharinic acid, although at high concentrations of calcium ion the yield of glucosaccharinic acid becomes comparable. In addition, the degradation of tetra-*O*-acetyl-2-hydroxy-D-glucal and the early stages of degradation of penta-*O*-acetyl-D-glucose, 2,3,4,6-tetra-*O*-acetyl-D-glucose, and penta-*O*-acetyl-*aldehydo*-D-glucose have been briefly studied.

In their study of the degradation of cellobiose by calcium hydroxide, Corbett and Kenner [1] found that the yield of acid estimated from the alkali consumed was greater than that determined from the acidity of the solution after removal of calcium ions by ion-exchange resin. Whereas the latter method gave a value close to the expected one of 2·6 equiv. for complete decomposition to acidic products (1 equiv. from the saccharinic acid produced by β-alkoxycarbonyl elimination, and 1·6 equiv. from the glucose thus formed [2]), the former gave a value between 3 and 4 equiv. It was therefore suggested that the yield of acid estimated by the ion-exchange resin method was the correct one, and that the difference

Table 1. *Degradation of cellobiose by saturated lime-water at* 25°.

Time (hr.)	Cellobiose decompd. (equiv.)	Monoses produced (equiv.)	Calc.*	Acid yield (equiv.) Back-titre method	Acid yield (equiv.) Resin method
1	0·00	0·00	0·00	0·01	0·00
2	0·01	0·01	0·01	0·02	0·01
3	0·02	0·02	0·03	0·04	0·02
4	0·02	0·04	0·02	0·05	0·04
6	0·05	0·06	0·05	0·09	0·07
10·5	0·17	0·18	0·17	0·20	0·17
24	0·44	0·45	0·44	0·51	0·50
31	0·54	0·53	0·55	0·67	0·64
46	0·67	0·64	0·71	0·89	0·86
53	0·73	0·65	0·86	0·98	0·94
71	0·82	0·72	0·99	1·13	1·09
144	0·84	0·69	1·09	1·29	1·25
240	0·90	0·74	1·17	1·44	1·41

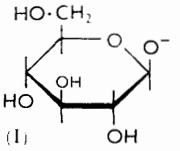
* Calc. by assuming that cellobiose decomposes to 1 equiv. of acid and 1 mol. of glucose which further decomposes to 1·6 equiv. of acid.

between the two methods was due to absorption of calcium hydroxide by undegraded cellobiose. This aspect of the degradation of cellobiose has now been re-investigated in detail.

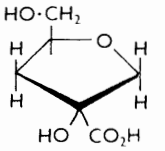
Examination of the resin method showed that results previously obtained were low because of lactonisation of saccharinic acids by the resin. When modified to eliminate lactone formation, the resin method gave yields of acid in agreement with those determined from the alkali consumed (Table 1). The observed excess of acid over the expected value of 2·6 equiv. is therefore real and is not due to absorption of calcium hydroxide. The directing influence of the substituent at the 4-position of the reducing glucose unit on the degradation of cellobiose to one molecule of saccharinic acid and one molecule of glucose is shown by the fact that, until the later stages of the reactions, the acid produced is

[1] Corbett and Kenner, *J.*, 1955, 1431.
[2] Kenner and Richards, *J.*, 1954, 1784.

approximately equal to the disaccharide decomposed. It therefore appears that degradation of the glucose formed from cellobiose is responsible for the excess of acid. This glucose will exist initially as the glucosyloxy-ion (I), which could possibly react with alkali to give more acid than does the normal complex mixture of ions which occurs in alkaline solutions of glucose. A direct proof of this cannot be achieved because of the difficulty of obtaining glucosyloxy-ions. However, since deacetylation of acetates produces the ion of the corresponding alcohol as an intermediate, a study has been made of the action of alkali on various glucose acetates.



(I)



(II)

Penta-*O*-acetyl-α-D-glucose underwent deacetylation, at rates dependent on its crystal size, when suspended in saturated oxygen-free lime-water (Fig. 1A) or in a suspension of calcium hydroxide (Fig. 1B). The rapid formation of acid did not cease when deacetylation was complete but continued until an acid yield of *ca.* 5·2 equiv. was reached; the rate then decreased to that found for glucose.[2] Penta-*O*-acetyl-β-D-glucose in saturated lime-water behaved in a similar manner. In contrast, the α-acetate in 0·05N- and 1·0N-sodium hydroxide underwent deacetylation followed by normal degradation (Fig. 1C and 1D, respectively). The continued rapid production of acid after deacetylation by lime-water is apparently due to the intermediate formation and subsequent degradation of the glucosyloxy-ion since penta-*O*-acetyl-*aldehydo*-D-glucose and 2,3,4,6-tetra-*O*-acetyl-β-D-glucose behaved normally (Figs. 2A and B, respectively). It appears that the glucosyloxy-ion is converted into the normal mixture of glucose ions within 2 hr. This explains the fall in rate of acid production to that observed for glucose.

FIG. 1. *Degradation of penta-*O*-acetyl-*D*-glucose by* (A) 0·045N-*lime-water*, (B) *suspension of lime*, (C) 0·050N-*sodium hydroxide, and* (D) 1·00N-*sodium hydroxide.*

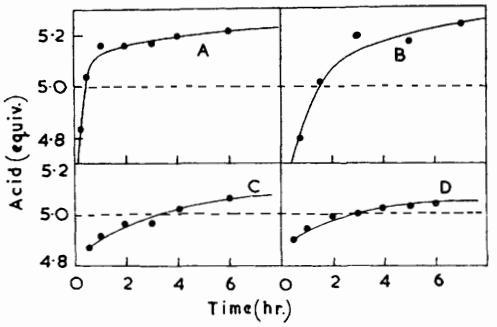
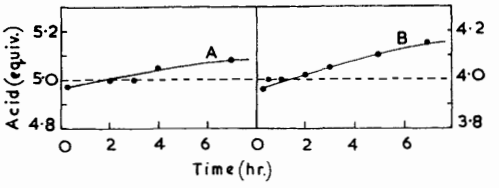


FIG. 2. (A) *Degradation of penta-*O*-acetylaldehydo-*D*-glucose by* 0·044N-*lime-water*. (B) *Degradation of* 2,3,4,6-*tetra*-O*-acetyl-*β*-*D*-glucose by* 0·041N-*lime-water*.



Paper-chromatography of the initial non-volatile acidic components of the lime-water degradation of penta-*O*-acetyl-D-glucose indicated that essentially only one acid (R_L 0·31; movement relative to lactic acid) was present. It resembled a reductone rather than a carboxylic acid, since its behaviour towards chromatographic sprays was very similar to that of ascorbic acid. Moreover, it was unaffected by alkali and therefore was not an ntermediate. In agreement with these quantitative experiments, the acid of R_L 0·31 could not be detected among the degradation products from penta-*O*-acetyl-D-glucose in 0·05N- or 1·0N-sodium hydroxide, or those from penta-*O*-acetyl-*aldehydo*- and tetra-*O*-acetyl-D-glucose in lime-water. However, it was formed from cellobiose by lime-water.

The acid was obtained only in very small amounts contaminated with glucometasaccharinic acid and chromatographically slow-moving acids.

The neutral products first formed by lime-water from penta-*O*-acetyl-D-glucose reacted slowly with acidic 2,4-dinitrophenylhydrazine to give a complex mixture from which was isolated a compound A. Since this was not obtained similarly from glucose it was apparently derived from a compound resulting from the glucosyloxy-ion. Under similar conditions, lactose should then give an analogous dinitrophenylhydrazine derivative, provided that the $C_{(4)}$ centre of the galactose moiety does not lose its asymmetry. Such a compound, B, was indeed isolated.

The constants of compound A are similar to those of the dinitrophenylhydrazine derivative of 2-hydroxy-D-glucal,[3] and on admixture there was no depression of the albeit indefinite melting point. The *X*-ray powder photographs of the two compounds differed (though this in turn could be due to different crystalline forms). The similarity is of interest since it has been suggested that 2-hydroxyglucal may be an intermediate in the alkaline degradation of glucose.[4] Accordingly, the acidic products from the alkaline

TABLE 2. *The degradation of 2-hydroxy-D-glucal tetra-acetate by lime-water at 25°: acid yields.*

0·009N-Lime-water		0·044N-Lime-water		0·044N-Lime-water		Suspension	
Time (hr.)	Equiv. of acid	Time (hr.)	Equiv. of acid	Time (hr.)	Equiv. of acid	Time (hr.)	Equiv. of acid
0·5	4·65	0·33	4·39	6	5·02	0·5	4·61
1	4·92	0·75	4·75	24	5·18	1	4·84
3	5·25	1	4·81	48	5·22	4	4·90
7·5	5·55	2	4·92			7	4·95
24	5·55					24	4·95

TABLE 3. *Degradation of 2-hydroxy-D-glucal tetra-acetate by lime-water at 25°: composition of acids.*

	0·011N-Lime-water	Suspension		0·011N-Lime-water	Suspension
Equiv. acids	5·26	5·07	Non-volatile acids (cont.):		
Volatile acids	4·00	4·03	lactone, R_L 0·67 ...	0·18	0·08
Non-volatile acids:			lactone, R_L 0·45 ...	0·15	0·25
lactic	0·02	0·02	lactone, R_L 0·05 ...	0·06	0·03
dihydroxybutyric ...	0·11	0·05	acid, R_L 0·51	0·42	0·18
glycollic	0·04	0·03	acid, R_L 0·31	0·18	0·24
lactone, R_L 0·82 ...	0·06	0·03	acid, R_L 0·05	0·04	0·03

degradation of 2-hydroxyglucal tetra-acetate have been briefly studied. Degradation occurs very readily (Table 2: the difference in rates is probably due to differences in crystal size, as observed for penta-*O*-acetylglucose), but the final yield of acid appears to vary inversely with the strength of alkali. An approximate analysis of the exceedingly complex mixture of acids was made by thick-paper chromatography, but many of the products could not be identified (Table 3). The main component was an acid of R_L 0·51 which could not be completely separated from an acid of R_L 0·31. The former corresponds to the acid, probably tetrahydro-3-hydroxy-5-hydroxymethylfuran-3-carboxylic acid[5] (II), produced by the alkaline degradation of 2-hydroxycellobial. Because this acid of R_L 0·31 does not react instantaneously with permanganate and also from analyses of the acidic degradation products of glucose (see below), it is concluded that 2-hydroxyglucal is not an intermediate in the alkaline degradation of glucose. Consequently compound A is not the 2,4-dinitrophenylhydrazine derivative of 2-hydroxy-D-glucal.

[3] Corbett, *J.*, 1959, 3213.
[4] Kusin, *Ber.*, 1936, **69**, 1041.
[5] Corbett and Kidd, *J.*, 1959, 1594.

The small amount of acid of R_L 0·31 produced (presumably) from glucosyloxy-ions (Fig. 1) cannot explain the excess of acid produced from the glucose moiety of cellobiose. In the cellobiose experiments the strength of the lime-water fell to *ca.* 0·01N before degradation of glucose became appreciable. As this was a quarter of the concentration used in the comparable experiment with glucose,[2] the effect of alkali concentration on the yield of acid from glucose has been studied.

Glucose was degraded with lime-water of varying strength, and the yields of acid were plotted against amounts of hexose decomposed (as measured by fall in reducing power). Extrapolation of the resulting straight lines gave values for complete decomposition. The results (Fig. 3 and Table 4) show that the total yield of acid is inversely related to the initial concentration of the lime-water and is independent of the glucose concentration.

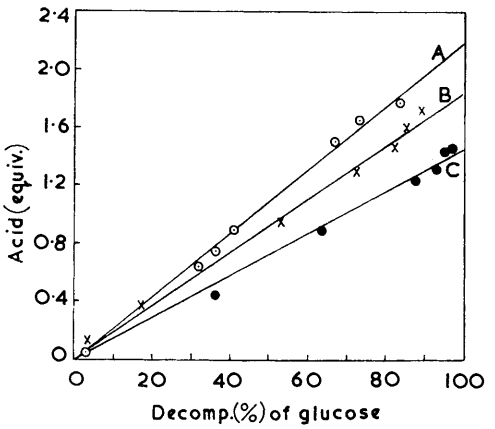


FIG. 3. *Degradation of glucose by* (A) 0·013N-*lime-water*, (B) 0·025N-*lime-water, and* (C) *suspension of lime.*

Further, this inverse relation is not peculiar to lime-water, but applies also to barium and sodium hydroxides (Table 4). From experiments in which the ionic strength of sodium hydroxide solutions was varied, it was shown that the yield of acid is inversely related to hydroxyl-ion concentration, and is independent of ionic strength (Table 4). Galactose, fructose, and xylose (Table 4) also show this inverse relation of yield of acid to alkali strength.

Since a number of competing reactions are involved in the alkaline degradation of unsubstituted sugars, one or more of these must be preferentially accelerated or retarded when the strength of alkali is decreased. In contrast, the degradation as a whole is catalysed by calcium ions, the half-life for glucose in 0·046N-lime-water and -sodium hydroxide being 50 and 350 hr., respectively. This is in agreement with the work of Kenner and Richards on substituted sugars.[6] However, with unsubstituted sugars the Lobry de Bruyn–Alberda van Ekenstein transformation is more important than with substituted sugars because degradation is more rapid in the latter case. It has been found that calcium ions have no influence on this transformation since glucose in both saturated lime-water and 0·04N-sodium hydroxide gives an equilibrium mixture of glucose 66%, fructose 33%, and mannose 1%.[7]

In order that the effect of hydroxyl and calcium ions might be investigated in more detail, the acidic degradation products of glucose with 0·01N-calcium hydroxide, a suspension of calcium hydroxide, and 0·05N-sodium hydroxide were analysed (Table 5). The non-volatile acids were separated by paper chromatography, but as the percentage elution from the paper was only of the order of 50%, the accuracy of the results is low. Nevertheless, there are marked differences in the composition of the complex mixtures of

[6] Kenner and Richards, *J.*, 1957, 3019.
[7] Cf. Speck, *Adv. Carbohydrate Chem.*, 1958, **13**, 63.

acids. Thus, substantially less of the unknown acid of R_L 0·48 is formed in 0·05N-alkali than in 0·01N-alkali. Its structure has not been determined because it could not be obtained pure, but from its chromatographic properties it appears to be identical with an acid obtained from xylose but different from the acid of R_L 0·51 obtained from 2-hydroxyglucal.

TABLE 4. *Degradation of monosaccharides by alkali at 25°.*

Alkali	Alkali/glucose (equiv./mole)	Salt added	Ionic strength	Total acid (equiv./mole)
		Glucose		
$Ca(OH)_2$				
Suspension	—	—	*	1·5
0·046	2·2	—	*	1·6
0·025	2·3	—	*	1·8
0·013	2·3	—	*	2·2
0·013	1·0	—	*	2·0
0·013	3·5	—	*	2·1
		$CaCl_2$		
0·013	—	0·014M	*	2·0
0·015	—	0·042M	*	2·0
0·025	—	0·024M	*	1·8
$Ba(OH)_2$				
0·044	—	—	*	1·7
0·020	—	—	*	2·6; 2·4
NaOH				
0·039	—	—	0·039	1·7
0·015	—	—	0·015	2·5; 2·3
		NaCl		
0·015	—	0·029M	0·044	2·6
0·015	—	0·076M	0·091	2·3
		$NaClO_4$		
0·015	—	0·035M	0·050	2·3
		$CaCl_2$		
0·045	—	0·022M	*	1·7
		Fructose		
$Ca(OH)_2$				
0·045	—	—	*	1·7
0·013	—	—	*	2·1
		Galactose		
0·047	—	—	*	1·5
0·038	—	—	*	1·6
0·011	—	—	*	1·8
0·009	—	—	*	2·1
		Xylose		
Suspension	—	—	*	1·4
0·010	—	—	*	1·8

* Not calculated.

TABLE 5. *Acidic products from the alkaline degradation of glucose at 25°.*

	Equiv./mole		
	0·01N-$Ca(OH)_2$	Satd. $Ca(OH)_2$	0·05N-NaOH
Total	2·0	1·6	1·6
Formic	0·58	0·10	0·18
Other volatile acids	0·24	0·10	0·18
Lactic	0·38	0·54	0·46
Unknown acid R_L 0·48	0·18	<0·01	0·03
Glycollic + acid of R_L 0·65	0·10	0·10	0·08
Dihydroxybutyric	0·10	0·13	0·08
Lactones, R_L 0—0·3	0·04	0·14	0·06
Lactone, R_L 0·43	0·02	0·03	0·08
D-Glucometasaccharinolactone	0·16	0·11	0·19
D-Glucoisosaccharinolactone	0·04	0·08	0·05
Lactone R_L 0·65	0·08	0·10	0·08
D-Glucosaccharinolactone	0·04	0·16	0·06

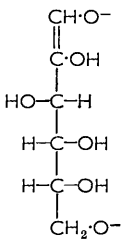
The most significant feature of Table 5 is the increase in the yield of formic acid in the case of dilute alkali. This is the main cause of the increase in yield of total acid. Recently, it has been shown that in sodium hydroxide solution, 3-dehydro-4-deoxy-D-fructose, an intermediate in the degradation of 4-substituted hexoses, undergoes fragmentation to give 0·83 equiv. of formic acid per equivalent of glucoisosaccharinic acid:[8] this does not explain the difference in yield of volatile acid from glucose in saturated and 0·01N-lime-water because of the very low yield of isosaccharinic acid (Table 5). Therefore, formic acid must

[8] Machell and Richards, *J.*, 1960, 1932.

also be produced by a reaction of unknown nature which is inversely dependent upon hydroxyl-ion concentration. Since the yield of 6-carbon saccharinic acids is high, a large percentage of the glucose must be degraded to 1 equiv. of acid per mole. This means that the yield of >2 equiv. of total acid per mole can be accounted for only by reactions producing 3 or more equiv. of acid. These reactions must involve extensive oxidation–reduction, since the systems studied were oxygen-free.

The effect of alkali on the nature of the saccharinic acids has also been determined. From paper chromatography (Table 5) it is apparent that the composition of lactonisable acids produced from glucose by 0·01N-lime-water and 0·05N-sodium hydroxide is approximately the same and that the major acid is glucometasaccharinic acid. With saturated lime-water, however, glucosaccharinic acid becomes of considerable importance, and its yield is comparable with that of glucometasaccharinic acid.

It is generally believed that the predominant saccharinic acid produced by the action of sodium hydroxide on glucose is glucometasaccharinic acid whereas that from calcium hydroxide is glucosaccharinic acid.[9] The evidence now presented suggests that glucometasaccharinic acid is a major product in both cases, as would be expected from the β-hydroxycarbonyl mechanism of the formation of saccharinic acids. However, when the calcium-ion concentration becomes appreciable, glucosaccharinic acid is formed by a different mechanism involving fragmentation and recombination.[10] This necessitates revising the explan-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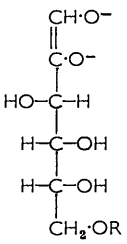
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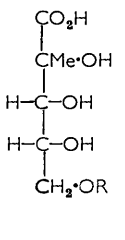
(V)

ation of the formation of 6-*O*-substituted glucometasaccharinic acids by the action of lime-water upon melibiose [11] and 6-*O*-methylglucose.[12] It was believed that because substitution of the primary hydroxyl group prevents formation of the ion (III), which leads to saccharinic acid, the ion (IV) is formed, giving rise to a metasaccharinic acid. In the light of the present work it is unnecessary to postulate the formation of doubly charged ions since the main saccharinic acid expected from a 6-*O*-substituted glucose is of the meta-type. Moreover, substitution of the primary hydroxyl group would be likely to prevent the formation of a 5-*O*-substituted saccharinic acid (V) by a fragmentation-recombination process.

Experimental

Qualitative Paper Chromatography.—Separations were made on Whatman No. 1 filter paper, development being with solvent (*a*) ethyl acetate–acetic acid–water (10 : 1·3 : 1) or (*b*) ethyl acetate–pyridine–water (8 : 2 : 1). Components were detected by the sprays (*a*) B.D.H. "4·5" indicator,[13] (*b*) sodium periodate–permanganate,[14] and (*c*) hydroxylamine–ferric chloride.[15]

Degradation of Cellobiose with Saturated Lime-water.—The degradation of cellobiose (4·748 g.)

[9] Sowden, *Adv. Carbohydrate Chem.*, 1957, **12**, 75.
[10] Sowden, Blair, and Kuenne, *J. Amer. Chem. Soc.*, 1957, **79**, 6450.
[11] Corbett and Kenner, *J.*, 1954, 3281.
[12] Kenner and Richards, *J.*, 1956, 2916.
[13] Nair and Muthe, *Naturwiss.*, 1956, **43**, 106.
[14] Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.
[15] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.

in saturated, oxygen-free lime-water (500 ml.) at 25° was followed as described by Corbett and Kenner [1] except that the following method was used for estimation of acids by the resin method. Aliquot portions (10 ml.) were stirred with Amberlite resin IR-120(H) (1 ml.) and phenolphthalein (1 drop) until the colour was discharged, and then for a further 10 min. The solution was filtered and the resin washed with water (5 × 5 ml.). The combined filtrate and washings were titrated with 0·01N-sodium hydroxide. A blank estimation was necessary. Results are given in Table 1. When a solution of calcium α-isosaccharinate was similarly treated, it was shown that the extent of lactone formation was less than 2·5%.

Alkaline degradation of Various Glucose Acetates.—The acetates (*ca.* 0·8 g.) were shaken in oxygen-free alkali (250 ml.) until dissolution had been attained. Samples (25 ml.) were removed periodically, acidified with 0·01N-sulphuric acid, and back-titrated with 0·05N-sodium hydroxide. Further samples, after being stirred with Amberlite resin IR-120(H), were concentrated under reduced pressure and chromatographed in solvent *a*. Sprays *a*, *b*, and *c* revealed that during the first stages of reaction of penta-*O*-acetyl-α- and -β-D-glucose with lime-water the main acidic product had R_L 0·31. It reacted instantaneously with spray *b* and with potassium permanganate alone. Slow-moving components (R_L 0·02—0·14) and traces of metasaccharinolactone (R_L 0·51) were also detected. Only slow-moving components and metasaccharinolactone were detected amongst the initial degradation products of penta-*O*-acetyl*aldehydo*- and 2,3,4,6-tetra-*O*-acetyl-D-glucose with lime-water and penta-*O*-acetyl-D-glucose with 0·045N- and 1·0N-sodium hydroxide. Quantitative results are given in Fig. 1 and 2.

Neutral Products from the Alkaline Degradation of Penta-O-*acetyl*-D-*glucose*, D-*Glucose, and Lactose.*—The sugar (50 g.) was shaken for 18 hr. with a suspension of calcium hydroxide (10 g.) in water (1 l.), and then the suspension was saturated with carbon dioxide, boiled, filtered, and concentrated under reduced pressure to a syrup. This was dissolved in a saturated solution (300 ml.) of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid and kept at room temperature for 7 days. The precipitate was centrifuged off, washed with water until free from acid, and dried. It was fractionally crystallised from ethanol, chloroform, and acetone to give from penta-*O*-acetyl-D-glucose compound A, m. p. 242—244° [Found: C, 41·2; H, 3·2; N, 21·2%; *M* (Rast) 522], and from lactose compound B, m. p. 214—219° (Found: C, 41·2; H, 3·2%). D-Glucose gave several compounds, none of which corresponded to the above.

Alkaline Degradation of Tetra-O-*acetyl*-2-*hydroxy*-D-*glucal.*—(a) *Rate of degradation.* Samples of the acetate were degraded with 0·009N- and 0·044N- and a suspension of calcium hydroxide under conditions similar to those described for the glucose acetates. In the last two experiments, an intense yellow colour was produced within 15 min. and disappeared in 0·5—1·5 hr. Results are given in Table 2.

(b) *Quantitative analysis of acid products.* (i) By 0·011N-lime-water. 2-Hydroxy-D-glucal tetra-acetate (0·649 g.) was treated with the alkali (1·2 l.) for 20 hr. at room temperature. The solution was stirred with excess of Amberlite resin IR-120(H) until free from calcium ions, and then diluted to 1·5 l. Samples (10 ml.) required 1·38 ml. of 0·05N-sodium hydroxide for neutralisation to phenolphthalein. Addition of further 0·05N-sodium hydroxide (3 ml.) followed by back-titration after 1 hr. indicated the absence of lactones. The yield of acid was therefore 5·26 equiv. per mole. The solution (1·460 l.) was concentrated under reduced pressure, and samples (10 ml.) of the distillate required 1·10 ml. of 0·05N-sodium hydroxide for neutralisation (*i.e.*, 3·97 equiv. per mole, 99·3% of theory for acetic acid). Samples of the residue were quantitatively analysed by the method described for glucose below; the results are given in Table 3.

(ii) By a suspension of lime. The acetate (0·592 g.) was similarly treated with a suspension of calcium hydroxide (5 g.) in water (1·2 l.). After 20 hr. the suspension was filtered, freed from calcium ions, and diluted to 1·5 l. Samples (10 ml.) required 1·21 ml. of 0·05N-sodium hydroxide for neutralisation. Addition of further 0·05N-sodium hydroxide (3 ml.) followed by back-titration indicated the absence of lactones. The yield of acid was therefore 5·07 equiv. per mole. The solution (1·470 ml.) was concentrated under reduced pressure and samples (10 ml.) of the distillate (1·450 l.) required 0·96 ml. of 0·05N-sodium hydroxide for neutralisation (*i.e.* 4·04 equiv. per mole, 101% for acetic acid). Samples of the residue were quantitatively analysed as above; results are given in Table 3.

Alkaline degradation of Various Monoses: Acid Yields.—The initial ratio of alkali to monose was, as far as possible, 2·3 equiv. per mole. At intervals aliquot portions of the reaction mixture were removed for analysis. Acid production was estimated by back-titration, except for the

experiments with a suspension of lime where the resin method as described for the cellobiose experiments was used. Monose consumption was measured by the fall in reducing power towards potassium ferricyanide.[16] Results are given in Table 4.

Lobry de Bruyn–Alberda van Ekenstein Transformation of Glucose.—Solutions of D-glucose (4·5 g.) in oxygen-free saturated lime-water (25 ml.) and in 0·04N-sodium hydroxide (25 ml.) were kept at 25° until their optical rotations were constant (500—800 hr.). After treatment with excess of Amberlite resin IR-120(H), the solutions were submitted to quantitative paper chromatography with solvent *b*. By this method the mixture was separated into only two bands, the slower being glucose and the faster fructose plus mannose. Total sugars in each band were estimated by Hagedorn and Jensen's method,[16] and fructose in the faster fraction by Bell's method.[17] In both cases the equilibrium mixture contained glucose 66%, fructose 33%, and mannose 1%.

Alkaline degradation of Glucose: Analysis of Acidic Products.—The acidic degradation products of glucose from 0·05N-sodium hydroxide and 0·01N- and a suspension of calcium hydroxide were determined by the same method. Details for the experiment with 0·05N-sodium hydroxide are given as an example. Glucose (4·026 g.) in oxygen-free alkali (1 l.) was kept at 25° until *ca.* 80% degradation had occurred.

(a) *Volatile acids.* Aliquot portions (100 ml.) of the reaction mixtures were stirred with Amberlite resin IR-120(H) (10 ml.) for 20 min., then filtered, and washed with water (5 × 10 ml.). The combined filtrate and washings were evaporated and the volatile acids determined by Richards and Sephton's method.[18] Formic acid was oxidised by refluxing a sample of the volatile acids with an excess of mercuric oxide for 2·5 hr., the residual acidity being determined by titration to thymolphthalein.

(b) *Non-volatile acids.* An aliquot portion (400 ml.) of the reaction mixture was stirred with Amberlite resin IR-120(H) (70 ml.) for 2 hr., then filtered, and the resin was washed with water (4 × 50 ml.). The combined filtrate and washings were concentrated to a solution containing 0·104 milliequiv. of acid plus lactone per ml. Samples (10 ml.) were chromatographed on Whatman No. 3MM paper (12 in. wide) in solvent *a*. After the solvent had been allowed to evaporate (steaming was necessary in dry atmospheres), guide strips were sprayed with spray *a* to locate the position of the acids. The entire chromatograms were then cut into strips, and the acids and lactones were eluted with water (75—100 ml.). The acidity of the eluants was determined by direct titration. Further 0·01N-sodium hydroxide (2 ml.) was added and the solution kept for 1 hr. in an atmosphere of nitrogen. The lactone concentration was determined from the excess of alkali. Determinations were made in triplicate.

(c) *Lactonisable acids.* An aliquot portion (20 ml.) of the concentrated solutions used for the above analyses was diluted to 30 ml., stirred with Amberlite resin IRA-400 (CO_3) (1·5 ml.) until it was of pH 4·0, and then filtered. The resin was washed with water (4 × 10 ml.) and the combined filtrate and washings were diluted to 100 ml. Titration of duplicate samples (5 ml.) indicated that the lactones were recovered in 96% yield whereas 95·5% of the acids were removed. The remaining solution was concentrated (to *ca.* 5 ml.) and chromatographed on Whatman No. 3MM paper as described above except that the lactones on the guide strips were detected by spray *c*. Estimations were duplicated.

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