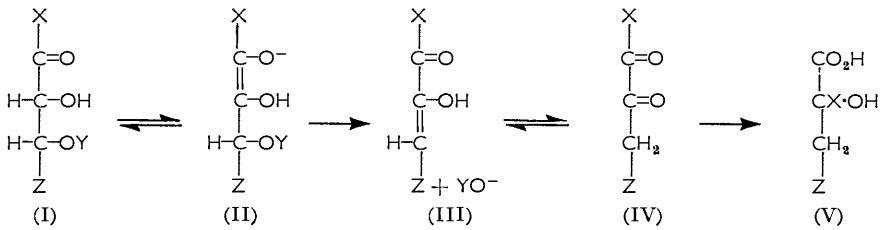
## 384. *Mechanism of Saccharinic Acid Formation. Part I. Competing Reactions in the Alkaline Degradation of* 4-O-*Methyl*-D-*glucose, Maltose, Amylose, and Cellulose.*

By GREVILLE MACHELL and G. N. RICHARDS.

In the alkaline degradation of the 4-*O*-substituted glucose derivatives, 4-*O*-methyl-D-glucose, maltose, amylose, and cellulose, calcium hydroxide favours the formation of D-glucoisosaccharinic acid whereas, in sodium hydroxide, fragmentation predominates. Under certain conditions, the latter reactions yield mainly βγ-dihydroxybutyric and glycollic acid, which possibly arise from the αβ-diketone postulated as precursor of D-glucoisosaccharinic acid.

PREVIOUS work [1] on the alkaline degradation of partially substituted reducing sugars [2a] has resulted in formulation of a general mechanism of saccharinic acid formation (I ⟶ V), which confirms Isbell's hypotheses.[2] There is evidence that both inductive and mass-law effects [3] favour the elimination (II ⟶ III) of an ether-linked substituent (OY) relative to that of a hydroxyl group in aqueous alkali. Thus it was observed that alkaline degradation of suitably substituted reducing sugars often produced high yields of a particular type of saccharinic acid, whereas similar treatment of the parent sugar yielded a complex mixture of saccharinic acids. Such generalisations, however, have been based almost entirely on reactions in lime-water at room temperature, whereas a recent study of the action of 0·5N-sodium hydroxide at 100° on cellulose (which may be regarded as a 4-*O*-substituted glucose) has indicated the extensive occurrence of several competing reactions, possibly resulting from fragmentation of an intermediate such as (IV).[4] Further, a different type of mechanism may be responsible [5] for the formation of D-glucosaccharinic acid from D-[1-$C^{14}$]mannose. Further confirmation of the reaction scheme (I ⟶ V) will therefore be sought, with the particular object of investigating the properties of the postulated αβ-dicarbonyl intermediates (IV) and the role of calcium in this type of reaction.

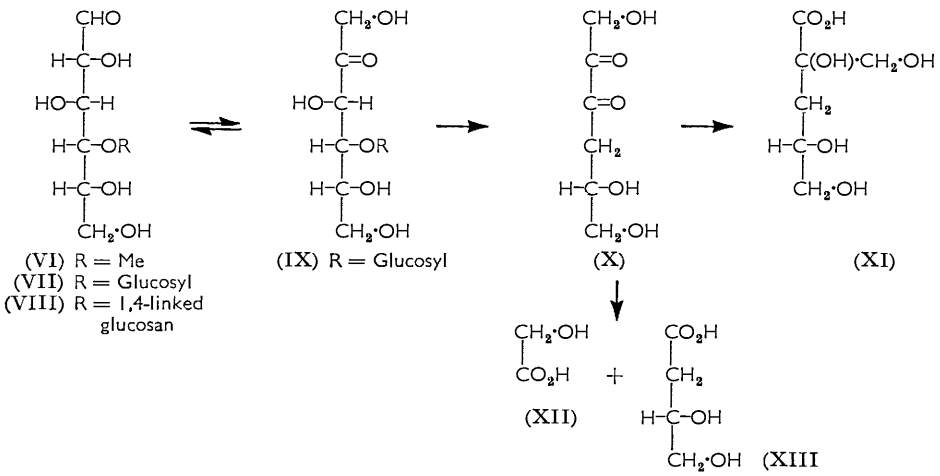



In accordance with the above generalisations, it was known that D-glucoisosaccharinic acid (XI) is the major product of degradation, by lime-water, of several 4-*O*-substituted D-glucose derivatives, including the 1,4-linked glucans, *viz.*, disaccharides,[6] 4-*O*-methyl-D-glucose,[7] amylose,[8] and cellulose.[4] In view of the relatively slow formation of saccharinic acid in these cases (compared with, *e.g.*, 3-*O*-methyl-D-glucose [9]), this type of system

[1] Kenner and Richards, *J.*, 1957, 3019 and earlier references.
[2] (*a*) Cf. Raymond, "Organic Chemistry," ed. Gilman, Wiley and Sons Inc., New York, 1942, Vol. II, p. 1648; (*b*) Isbell, *J. Res. Nat. Bur. Stand.*, 1944, **32**, 45.
[3] Cf., *e.g.*, Ingold, "Structure and Mechanism in Organic Chemistry," Bell and Sons Ltd., London, 1953, p. 360.
[4] Richards and Sephton, *J.*, 1957, 4492.
[5] Sowden and Kuenne, *J. Amer. Chem. Soc.*, 1953, **75**, 2788; Sowden, Blair, and Kuenne, *ibid.*, 1957, **79**, 6450.
[6] Corbett and Kenner, *J.*, 1955, 1431, and earlier references.
[7] Kenner and Richards, *J.*, 1955, 1810.
[8] Kenner and Richards, *Chem. and Ind.*, 1954, 1483; Machell and Richards, *J.*, 1958, 1199.
[9] Kenner and Richards, *J.*, 1954, 278.

appeared most suitable for a study of competing reactions. It has been shown [1] that lime-water accelerates the alkaline degradation of 3-*O*-methyl-D-glucose, and the present results indicate a similar effect in the early stages of alkaline degradation of 4-*O*-methyl-D-glucose (VI) (Table 2), maltose (VII) (Table 3), and maltulose (IX) (Table 4). In addition, in the later stages of the reaction a significantly greater yield of acid was obtained in sodium hydroxide than in lime-water. In the degradation of 4-*O*-methyl-D-glucose by sodium hydroxide the final yield of acid was considerably greater than 1·0 equiv. per mole. It therefore seemed possible that calcium was catalysing some stage in the formation of the isosaccharinic acid (VI ⟶ XI) at the expense of fragmentation which might yield more than one equivalent of acid.

Qualitative paper chromatography of the acids produced in the early stages of such degradations in sodium hydroxide showed the presence of considerable amounts of glycollic and a dihydroxybutyric acid. The former was isolated by paper chromatography and identified as the 4-bromophenacyl glycollate. The dihydroxybutyric acid reacted with sodium metaperiodate much more rapidly than αγ-dihydroxybutyric acid and was also isolated by paper chromatography and shown to be D-βγ-dihydroxybutyric acid by comparison with an authentic sample prepared from D-βγ-dihydroxybutyraldehyde. This acid appears to be identical with the dihydroxybutyric acid obtained by Glattfeld *et al.*[10] by aeration of an alkaline solution of maltose.




The alkaline degradation of several 4-*O*-substituted glucose derivatives (VI—VIII) was next investigated in more detail by determining the relative yields of the predominant acids produced under various conditions (see Table 1). All these results, except for formic acid, were obtained by a quantitative paper chromatographic method in which the recovery of an acid is dependent on its concentration on the paper (cf. ref. 11). The resultant correction factors have been applied as far as possible, but the results of Table 1 are subject to an error of 10%. Certain generalisations are, however, possible.

It is evident that the presence of calcium favours formation of isosaccharinic acid. We have already postulated [1] the catalysis by $(CaOH)^+$ of the elimination (II ⟶ III) in alkaline degradation of 3-*O*-alkyl-D-glucoses. In the present case, however, it was possible to isolate a small amount of an intermediate, tentatively identified as the diketone (X), by action of sodium hydroxide on maltose. This product rapidly yielded D-gluco-isosaccharinic acid on treatment with lime-water, and was only detected in traces in the

[10] Glattfeld and Hanke, *J. Amer. Chem. Soc.*, 1918, **40**, 987; but cf. Glattfeld and Miller, *ibid.*, 1920, **42**, 2314.

[11] Whistler, Chang, and Richards, *J. Amer. Chem. Soc.*, 1959, **81**, 3133, 4058.

degradation of maltose by lime-water. It seems possible, therefore, that specific cation-catalysis of the rearrangement (X ⟶ XI) may also occur and this possibility will be further investigated.

In each case reported in Table 1, alkaline degradation in sodium hydroxide solution yields mainly products of less than six carbon atoms, which we term fragmentation products. Among this type of product, D-βγ-dihydroxybutyric acid predominates at low temperatures and it seems possible that it is associated with the formation of glycollic acid, the two acids arising by scission of six-carbon units. The product most likely to undergo such scission is the postulated intermediate (X), and the alkaline degradation of this and of related αβ-diketones will be further investigated. The dihydroxybutyric acid is usually obtained in excess of glycollic acid; this disparity is greater than is apparent from Table 1 since only the hydroxybutyrolactone was determined and the true yield of the dihydroxybutyric acid will be greater by the amount occurring as the free acid on the paper chromatograms.

TABLE 1. *Acids (yields as percentage of total acid) formed in alkaline degradation of 4-O-substituted glucose derivatives.*

| Conditions | Substrate | Formic (%) | D-Glucoisosaccharinic (%) | Glycollic (%) | Lactic (%) | β-Hydroxy-γ-butyrolactone * |
|---|---|---|---|---|---|---|
| 0·04N-Lime-water; 25° | Maltose | 2 | 89 | — | — | — |
| | Cellulose | 5 | 88 | — | — | — |
| 0·04N-Lime-water; 100° | 4-*O*-Methyl-D-glucose | 12 | 63 | × | × | × |
| | Cellulose | <9 | 65 | × | 2 | × |
| | Amylose | 12 | 73 | × | 3 | × |
| 0·05N-NaOH; 25° | Maltose | 11 | 13 | 23 | — | 33 |
| | Cellulose | 7 | 20 | 22 | — | 25 |
| 0·5N-NaOH; 100° | 4-*O*-Methyl-D-glucose | 32 | 32 | × | × | × |
| | Cellulose | 36 | 30 | 9 | 6 | 8 |
| | Amylose | 35 | 24 | × | 6 | × |

× Not determined. — Not detected. * Corresponding free acid not determined.

Table 1 also shows that more formic acid is produced in concentrated sodium hydroxide at higher temperatures, apparently at the expense of dihydroxybutyric and glycollic acid. Formation of lactic acid is also favoured by higher temperatures, in accordance with previous observations on degradation of cellulose.[4,12]

## EXPERIMENTAL

The following solvents and sprays were used for paper chromatography with Whatman No. 1 paper at 25°: solvents A,[13] ethyl acetate–acetic acid–water (10 : 1·3 : 1); B,[14] butan-1-ol–pyridine–water (6 : 4 : 3); C,[15] butan-1-ol–ethanol–water (4 : 1·1 : 1·9). Sprays *a*,[16] sodium metaperiodate–potassium permanganate; *b*,[17] silver nitrate–sodium hydroxide; *c*,[15] naphtharesorcinol; *d*,[4] diphenylamine; *e*,[18] hydroxylamine–ferric chloride; *f*,[19] B.D.H. "4·5" indicator; *g*,[15] *p*-anisidine hydrochloride. $R_L$ refers to lactic acid.

*Effect of Calcium in Alkaline Degradation of 4-O-Methyl-D-glucose.*—A solution of 4-*O*-methyl-D-glucose [7] (0·194 g. ± 0·001 g.) in oxygen-free 0·1N-sodium hydroxide (25 ml.) was diluted to 50 ml., with an oxygen-free aqueous solution of the appropriate amount of calcium chloride. The solution was kept at 25° ± 0·1° and at intervals aliquot portions (4 ml.) were added to 0·05N-hydrochloric acid (5 ml.) and immediately titrated with 0·01N-sodium hydroxide

[12] Corbett and Richards, *Svensk Papperstidn.*, 1957, **60**, 791.
[13] Richtzenhain and Moilanen, *Acta Chem. Scand.*, 1954, **8**, 704.
[14] Jeanes, Wise, and Dimler, *Analyt. Chem.*, 1951, **23**, 415.
[15] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[16] Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.
[17] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[18] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[19] Nair and Muthe, *Naturwiss.*, 1956, **43**, 106.

to a transient phenolphthalein end-point. Nitrogen was bubbled through the reaction solution, and the flask was then stoppered after each withdrawal. The results are summarised in Table 2.

TABLE 2.

| Calcium added (equiv./mole) | $t_{0\cdot1}$ | $t_{0\cdot2}$ | $t_{0\cdot3}$ | $t_{0\cdot4}$ | $t_{0\cdot5}$ | Final acid yield (equiv./mole) |
|---|---|---|---|---|---|---|
| 0 | 45 | 70 | 85 | 105 | 125 | 1·31 |
| 2·0 | 12 | 27 | 45 | 65 | 86 | *ca.* 1·1 |

($t_x$ = time in hr. to produce $x$ equiv. of acid per mole.)

Paper chromatography of the non-volatile acidic products of these two reactions gave results almost identical with those described in detail below, for the degradation of maltose by sodium hydroxide and lime-water respectively.

*Alkaline Degradation of* 4-O-*Methyl*-D-*glucose at* 100°.—(a) *By lime-water.* Samples of 4-*O*-methyl-D-glucose (0·01 g.) were heated on the boiling-water bath with oxygen-free 0·04N-lime-water (3 ml.) for varying times, then cooled, 0·05N-hydrochloric acid (4 ml.) was added, and the solution titrated with 0·01N-sodium hydroxide. Some calcium hydroxide separated in the early stages of heating. Formation of acid was observed as follows: 0·90 (1 hr.), 0·94 (2 hr.), 1·02 (4 hr.), 1·05 (6 hr.), 1·11 (20 hr.) equiv./mole.

(b) *By sodium hydroxide.* 4-*O*-Methyl-D-glucose (0·015 g.) in oxygen-free 0·05N-sodium hydroxide (2 ml.) was heated at *ca.* 100° as above and acids determined as follows: 1·39 (1 hr.); 1·37 (3 hr.) equiv./mole.

*Determination of Relative Acid Yields from Degradation of* 4-O-*Methyl*-D-*glucose by Lime-water at* 100°.—(a) D-*Glucoisosaccharinic acid.* An oxygen-free aqueous solution of 4-*O*-methyl-D-glucose (0·785 g., in 30 ml.) was heated with calcium hydroxide (1 g.) on the boiling-water bath in an atmosphere of nitrogen with occasional shaking for 4 hr. After cooling, the solution was filtered, saturated with carbon dioxide, concentrated to *ca.* 10 ml., again filtered, and finally evaporated to dryness. An aqueous solution (5 ml.) of the residue was next treated with Amberlite resin IR-120(H) (7 ml.) at 60° for 10 min., and the solution was decanted, combined with washings (*ca.* 5 ml.), and centrifuged. The clear centrifugate was shown, by back-titration of the excess of alkali in an aliquot portion, to contain 0·355 milliequiv. of acid per ml. An aliquot portion of this solution (4·50 ml.) was transferred to Whatman No. 3 MM paper, and afte relution with solvent A, D-glucoisosaccharinic acid was determined as described earlier.[4] The acidic band contained 0·102 milliequiv. (corr.) and the lactone band 0·956 milliequiv. (corr.) of total acids, while the free acid in the latter corresponded to 0·074 milliequiv. (corr.). The yield of D-glucoisosaccharinic acid therefore corresponded to 63% of the total acid equivalents (see Table 1).

Qualitative paper chromatography of the acidic products in solvent A indicated that the minor acidic products observed [4] in the degradation of cellulose by sodium hydroxide were again produced, but in considerably smaller amount. In the present experiment, however, more of the unidentified acid ($R_L$ 0·34) which reacted rapidly with spray *a* was apparently formed than in the cellulose–sodium hydroxide system. The major component on the chromatogram again corresponded to D-glucoisosaccharinolactone ($R_L$ 0·58).

(b) *Volatile acids.* A solution of 4-*O*-methyl-D-glucose (0·0461 g.) in oxygen-free 0·03N-lime-water (20 ml.) was heated under nitrogen on the boiling-water bath for 6 hr., cooled, and passed through Amberlite resin IR-120(H) (2 ml.). An aliquot portion of the resulting acidic solution was distilled as described earlier [4] for formic acid determinations, and the distillate was found to contain 12% of the total acidity (see Table 1).

*Determination of Relative Acid Yields from Degradation of* 4-O-*Methyl*-D-*glucose by Sodium Hydroxide at* 100°.—(a) D-*Glucoisosaccharinic acid.* A solution of 4-*O*-methyl-D-glucose (0·657 g.) in oxygen-free 0·5N-sodium hydroxide (30 ml.) was heated under nitrogen on the boiling-water bath for 1 hr. After cooling, the solution was passed through Amberlite resin IR-120(H) (25 ml.) and excess of aqueous ammonia added to the eluate and washings, which were evaporated to dryness. An aqueous solution (5 ml.) of the residue was then treated with Amberlite resin IR-120(H) (7 ml.), and the proportion of D-glucoisosaccharinic acid determined as described above. 1·719 milliequiv. of acids were transferred to the paper, and the acid and lactone bands contained 0·10 and 0·52 milliequiv. (corr.) of the total acid respectively, while the latter included 0·081 milliequiv. of free acid. The yield of D-glucoisosaccharinic acid therefore [4] corresponded to 32% of the total acid equivalents (see Table 1).

(b) *Volatile acids.* A solution of 4-*O*-methyl-D-glucose (0·110 g.) in oxygen-free 0·5N-sodium hydroxide (5 ml.) was heated in nitrogen on the boiling-water bath for 1 hr. The volatile acids, determined as described above, corresponded to 36% of the total acidity, while determination of formic acid as described earlier [4] accounted for 32% of the total acidity (see Table 1).

*Alkaline Degradation of Maltose at* 25°.—(a) *In lime-water.* A solution of maltose monohydrate (1·712 g.) in oxygen-free 0·040N-lime-water (250 ml.) was kept at 25° while, at intervals, aliquot portions (10 ml.) were (i) run into 0·05N-hydrochloric acid (10 ml.) and immediately titrated with 0·01N-sodium hydroxide (phenolphthalein indicator), (ii) run on to freshly washed Amberlite resin IR-120(H) (1 ml.) to which a drop of phenolphthalein had been added. The resulting mixture was stirred at room temperature until the indicator was decolorised (1—2 min.), then for a further 2 min. and immediately filtered, and the resin was washed with water (3 × 5 ml.). The filtrate and washings were titrated with 0·01N-sodium hydroxide. Procedures (i) and (ii) gave identical results over the whole range of reaction studied, but use of larger amounts of resin or longer periods of contact with the resin caused appreciable lactonisation and consequent apparently low acid yields (cf. ref. 6). The results are expressed in Table 3.

Paper-chromatographic examination of the lime-water solution in solvent B, with sprays *b* and *c*, after deionisation with mixed Amberlite resins IR-120(H) and IR-B(OH), indicated the rapid formation of maltulose ($R_F$ 0·26), but glucose ($R_F$ 0·37) was not detected until after 1·5 hr. At the same time, very faint traces of the supposed intermediate (X) ($R_F$ 0·74) were detected. This compound (X) had $R_F$ 0·50 in solvent C and possibly corresponds to the major neutral product of the action of sodium hydroxide on cellulose.[4] The relative amount of glucose increased considerably with time, while that of compound (X) increased only slightly. At later stages of the reaction (*ca.* 24 hr.) traces were observed of a substance with the same $R_F$ (0·53) and reaction with spray *d* as 2-deoxy-D-ribose.

(b) *In* 0·05N-*sodium hydroxide.* A solution of maltose monohydrate (1·710 g.) in oxygen-free 0·05N-sodium hydroxide (250 ml.) was kept at 25° and examined as in the preceding experiment. Paper chromatography indicated that formation of maltulose was again rapid. Glucose was again detected after 1·5 hr. and increased progressively; but in this case more of the supposed intermediate (X) was present, and for the first 7 hr. of the reaction appeared to be of similar concentration to glucose, although at later stages the latter predominated. The neutral products at later stages of the reaction became more complex (see below).

*Alkaline Degradation of Maltulose at* 25°.—(a) *In lime-water.* A solution of maltose (0·333 g )

TABLE 3. *Alkaline degradation of maltose at* 25°.

| | 0·04N-Lime-water | | | | | 0·05N-Sodium hydroxide | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Acid produced | | | Acid produced | | Acid produced | | | Acid produced | |
| Time | (equiv./mole) | | Time | (equiv./mole) | | Time | (equiv./mole) | | Time | (equiv./mole) |
| (hr.) | (i) | (ii) | (hr.) | (i) | (ii) | (hr.) | (i) | (ii) | (hr.) | (i) | (ii) |
| 0·5 | 0·001 | 0·005 | 29·5 | 0·908 | 0·899 | 0·5 | 0 | — | 24 | 0·264 | — |
| 1·0 | 0·010 | 0·014 | 54 | 1·15 | — | 1·0 | 0 | — | 31 | 0·383 | — |
| 2·0 | 0·042 | 0·038 | 77 | 1·29 | — | 2·0 | 0 | — | 55 | 0·857 | 0·846 |
| 3·5 | 0·102 | — | 148 | 1·50 | 1·50 | 3·5 | 0 | — | 75 | 1·15 | — |
| 6·0 | 0·249 | 0·250 | 198 | 1·62 | — | 4·5 | 0·001 | — | 150 | 1·61 | — |
| 23 | 0·806 | — | | | | 7·0 | 0·018 | — | 198 | 1·93 | — |

TABLE 4. *Alkaline degradation of maltulose at* 25°.

| | 0·04N-Lime-water | | 0·05N-Sodium hydroxide | |
|---|---|---|---|---|
| Time (hr.) | $[\alpha]_D^{25}$ | Acid produced (equiv./mole) (i) | $[\alpha]_D^{25}$ | Acid produced (equiv./mole) (i) |
| 0·1 | +63° | 0·02 | +64° | 0·005 |
| 0·2 | — | 0·05 | — | 0·01 |
| 0·4 | +59 | 0·11 | +63 | 0·02 |
| 1·0 | +50 | 0·24 | +61 | 0·02 |
| 2·0 | +41 | 0·40 | +60 | 0·025 |
| 3·0 | +36 | 0·51 | +58 | 0·03 |
| 4·0 | +32 | 0·60 | +56 | 0·05 |
| 5·5 | +27 | 0·67 | +52 | 0·09 |
| 7·0 | +22·5 | 0·71 | +48 | 0·125 |

in oxygen-free 0·039N-lime-water (50 ml.) was kept at 25° and examined as described above for maltose. The results are expressed in Table 4.

(b) *In 0·05N-sodium hydroxide.* A solution of maltulose (0·333 g.) in oxygen-free 0·05N-sodium hydroxide (50 ml.) was treated as in the preceding experiment. Paper chromatography indicated more of the compound (X) in the early stages of the reaction (1—7 hr.) than in the preceding experiment.

*Acidic Products from Alkaline Degradation of Maltose at 25°.*—(a) *In lime-water.* A solution of maltose monohydrate (35·0 g.) in oxygen-free 0·04N-lime-water (5 l.) was kept at 25° for 3·5 hr., then saturated with carbon dioxide and concentrated to 200 ml. After filtration the solution was passed through Amberlite resin IR-120(H) (20 ml.) and stirred at room temperature overnight with De-acidite FF resin (25 g.; air dried; 200—400 mesh; low cross-linked, carbonate form).[20] The latter resin was next separated by filtration, washed continuously with water for 4 hr., and then eluted with N-aqueous ammonia (600 ml.). The eluate and washings were concentrated to 50 ml., and then passed through Amberlite resin IR-120(H) (20 ml.). An aliquot portion (100 ml. from 117 ml.) of the combined eluate and washings from this treatment was distilled for determination of formic and total volatile acids, and lactic acid was determined in a further sample, as described earlier.[4]

Paper chromatography in solvent A with sprays *a*, *e*, and *f*, of the non-volatile acids indicated that D-glucoisosaccharinolactone ($R_L$ 0·58) was the major product, but an unidentified acid ($R_L$ 0·34, reacting readily with spray *a*) and a lactone ($R_L$ 0·69) corresponding to a 3-deoxy-D-pentonolactone, were also readily detected.

The acidic residue from the distillation for determination of volatile acid was dissolved in water (10 ml.) and analysed for D-glucoisosaccharinic acid by paper chromatography as described above.

The amount of free acidity in the lactone band was similar to that observed with the authentic saccharinic acid and was considerably less than that observed in a similar reaction in sodium hydroxide (see below). Results of the above determinations are recorded in Table 1.

(b) *In 0·05N-sodium hydroxide.* A solution of maltose monohydrate (28·0 g.) in oxygen-free 0·05N-sodium hydroxide (4 l.) was kept at 25° for 6·5 hr., and the pH of the solution then reduced to 8 by stirring with Amberlite resin IR-120(H) (120 ml.). The resin was filtered off rapidly, and the filtrate evaporated to *ca.* 200 ml. under reduced pressure and passed through a column of Amberlite resin IR-120(H) (30 ml.) into a stirred suspension of Amberlite resin IRA-400(carbonate) (30 ml.). After overnight stirring, the resin was collected in a column, and the solution of neutral products rejected. Acids were then eluted from the resin with N-ammonium carbonate (2 l.) during 24 hr., and the eluate was evaporated to dryness at 70°/15 mm. to decompose the excess of eluant. The residue of ammonium salts was treated with Amberlite resin IR-120(H) (10 ml.) in a column, and the acidic effluent treated as described for the corresponding stage of the preceding reaction. Results for the yield of formic acid and other volatile acids are recorded in Table 1.

Paper chromatography of the non-volatile acids as in the preceding experiment indicated the presence of the following components: (i) D-glucoisosaccharinic acid ($R_L$ 0·19); (ii), (iii) unindentified acids ($R_L$ 0·32 and $R_L$ *ca.* 0·49); (iv) D-glucoisosaccharinolactone ($R_L$ 0·58); (v) βγ-dihydroxybutyric acid ($R_L$ 0·61); (vi) glycollic acid ($R_L$ 0·76); (vii) β-hydroxy-γ-butyrolactone ($R_L$ 1·04). There were also smaller amounts of material corresponding to the two 3-deoxy-D-pentonolactones ($R_L$ 0·70 and $R_L$ 0·78). A portion of the non-volatile acids was transferred to a Whatman No. 3 MM paper (56 × 61 cm.) developed with solvent A at 25° for 7 hr., and the components corresponding to glycollic acid and β-hydroxy-γ-butyrolactone were eluted. The former yielded 4-bromophenacyl glycollate, m. p. and mixed m. p. 143—145°, while from the latter was prepared brucine βγ-dihydroxybutyrate, m. p. 179—180°, mixed m. p. with authentic compound (see below) 177—178°, $[\alpha]_D^{17}$ −27·25° (Found: N, 5·2. Calc. for $C_{27}H_{34}O_8N_2$: N, 5·45%). From this brucine salt, after treatment with Amberlite resin IR-120(H), was prepared the corresponding phenylhydrazide; on crystallisation from ethanol–ethyl acetate, this compound showed m. p. and mixed m. p. (see below) 98—99° (Found: N, 13·0. Calc. for $C_{10}H_{14}N_2O_3$: N, 13·3%).

Quantitative analysis of the non-volatile acid mixture as previously described was carried out on a second portion of the mixture, and gave the results shown in Table 1.

*Periodate Oxidation of βγ-Dihydroxybutyric Acid.*—The preceding experiment was repeated

[20] Cf. Machell, *J.*, 1957, 3389.

and part of the eluate from the paper chromatogram, containing β-hydroxy-γ-butyrolactone (total acidity, 0·154 milliequiv. in 20 ml.), was treated with 0·1N-sodium hydroxide (2 ml.) at 50° for 10 min. The solution was cooled and neutralised with hydrochloric acid, 0·1M-sodium metaperiodate (5 ml.) was added, and the whole was diluted to 50 ml. The consumption of periodate at room temperature was determined by reaction with acidic potassium iodide and titration with sodium thiosulphate as follows: 0·95 (0·5 hr.), 1·07 (1·5 hr.), 1·67 (4·5 hr.), 1·82 (7 hr.), 2·7 (24 hr.) moles/equiv.

A further sample of the lactone solution (0·154 milliequiv. in 20 ml.) was neutralised as above, 0·1M-sodium metaperiodate (3 ml.) added, and the whole kept at room temperature for 30 min. The solution was then stirred with an excess of a mixture of the resins Amberlite IR-120(H) and De-acidite G(OH) for 30 min., filtered, and treated with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid (20 ml.). After being kept at 0° overnight the yellow precipitate was separated, washed with 2N-hydrochloric acid and then water, and dried (0·0249 g.). When recrystallised several times from ethanol, this material had m. p. 164—166° alone or in admixture with the formaldehyde derivative.

*Neutral Products of the Action of* 0·05N-*Sodium Hydroxide on Maltose.*—A solution of maltose monohydrate (7·10 g.) in oxygen-free 0·05N-sodium hydroxide (1 l.) was kept at 25° for 6·5 hr., passed through a column of Amberlite resin IR-120(H) (80 ml.) and concentrated to 100 ml. The resulting solution was stirred with Amberlite resin IRA-400(carbonate) (20 ml.) at room temperature for 1 hr. (final conductivity 3·5 μmho), then filtered and evaporated to dryness. Paper chromatography of the colourless syrupy residue (R) (7·00 g.) in solvent B, with sprays *b* and *c*, indicated the presence of maltose ($R_F$ 0·24, main component), maltulose ($R_F$ 0·26), glucose ($R_F$ 0·37), traces of fructose ($R_F$ 0·405) and psicose ($R_F$ 0·43), and the supposed intermediate (X) ($R_F$ 0·74, orange when heated with spray *c*).

A small amount of the last component was isolated in the pure state by chromatography of (R) in solvent B on Whatman No. 3 MM paper, and treated with saturated lime-water at room temperature for 1 hr. No neutral products were detected in the resulting solution and after treatment with Amberlite resin IR-120(H), chromatography in solvent A with sprays *e* and *f* indicated the presence of only D-glucoisosaccharinic acid ($R_L$ 0·19) and its lactone ($R_L$ 0·58).

*Acidic Products from Alkaline Degradation of Cellulose.*—(a) *In lime-water at* 25°. Hydrocellulose was prepared as described elsewhere;[4] the material (50 g.) was suspended in 0·04N-lime-water (1 l.) in the absence of oxygen at 25° for 9 days. Undissolved hydrocellulose was then filtered off, and the acidic products formed (4·5 milliequiv.) were isolated as indicated in previous sections. Paper chromatography of the acids in solvent A with sprays *e* and *f* revealed components corresponding to D-glucoisosaccharinic acid ($R_L$ 0·18) and the related lactone ($R_L$ 0·58). Determination of these, and the volatile components of the mixture, gave the results shown in Table 1.

(b) *In lime-water at* 100°. Hydrocellulose (10·6 g.) was heated at 100° with 0·04N-lime-water (500 ml.) for 1 hr. in nitrogen. The acidic products (9·16 milliequiv.) were isolated, and paper chromatography of the mixture as in (a) indicated the presence of the components reported in (a), but, in addition, an unknown acid ($R_L$ 0·30) (reacting rapidly with spray *a*), βγ-dihydroxybutyric acid ($R_L$ 0·60), glycollic acid ($R_L$ 0·76), lactic acid ($R_L$ 1·00), and β-hydroxy-γ-butyrolactone ($R_L$ 1·04) were detected. Smaller amounts of the two 3-deoxy-D-pentonolactones ($R_L$ 0·68 and 0·76) and an unknown lactone ($R_L$ 0·82) were also found. The proportions of the acids in the mixture were determined, and are reported in Table 1.

(c) *In* 0·05N-*sodium hydroxide.* Hydrocellulose (50 g.) was treated with 0·05N-sodium hydroxide (1 l.) at 25° for 16 days. Paper chromatography of the acidic products (3·68 milliequiv.) indicated the presence of D-glucoisosaccharinic acid ($R_L$ 0·18), D-glucoisosaccharinolactone ($R_L$ 0·57), βγ-dihydroxybutyric acid ($R_L$ 0·61), glycollic acid ($R_L$ 0·77), and β-hydroxy-γ-butyrolactone ($R_L$ 1·03). There were indications of smaller amounts of an unknown acid ($R_L$ *ca.* 0·45), the two 3-deoxy-D-pentonolactones ($R_L$ 0·68 and 0·76), and an unknown lactone ($R_L$ 0·83); lactic acid ($R_L$ 1·00) was not detected. The results of a partial quantitative analysis of the mixture of acids are given in Table 1.

(d) *In* 0·5N-*sodium hydroxide at* 100°. The experiment of Richards and Sephton[4] was repeated: Hydrocellulose (10 g.) was treated with 0·5N-sodium hydroxide (100 ml.) in the absence of oxygen at 100° for 30 min. Paper chromatography of the acidic products (14·45 milliequiv.) indicated the presence in the mixture of all the components reported under (c),

but also gave strong indications of lactic acid ($R_L$ 1·00). The proportions of the components in the mixture were determined and results are given in Table 1.

*Acidic Products from Alkaline Degradation of Amylose.*—(a) *In lime-water at* 100°. To a freshly prepared solution of amylose (1 g.) in water (100 ml.) was added solid calcium hydroxide (1 g.), and the mixture was then kept at 100° for 4 hr. in the absence of oxygen. Paper chromatography of the acidic products formed (1·86 milliequiv.) indicated that they were similar to those formed from cellulose under similar conditions; the proportions of the various acids are recorded in Table 1.

*Preparation of* D-(−)-βγ-*Dihydroxybutyraldehyde* (2-*Deoxy*-D-*tetrose*).—The method used was essentially that of Venner.[21] From D-xylose (50 g.) was obtained a mixture of calcium salts, which was degraded by the Ruff procedure with hydrogen peroxide (2 × 27 ml. portions) and ferric acetate (2·8 g.) (as catalyst). The filtered solution was then deionised by passage through successive columns of Amberlite resins IR-120(H) (500 ml.) and IR-45 (750 ml.). Evaporation of the solution under reduced pressure afforded a syrup (3·2 g.) which was examined by paper chromatography in solvent A. Spray *e* revealed lactones corresponding to the two 3-deoxy-D-pentonolactones, $R_L$ 0·69 and 0·77, and β-hydroxy-γ-butyrolactone, $R_L$ 1·03. The syrup was then dissolved in water (50 ml.) and stirred with De-Acidite FF (200—400 mesh; low cross-linked) (carbonate) (30 ml.) for 24 hr. to adsorb the lactones. After the resin had been filtered off, the solution was again evaporated to a syrup (1·8 g.), and shown by paper chromatography as above to contain only traces of lactone. Further chromatography in solvent B with spray *b* revealed material of $R_F$ 0·67 corresponding to authentic D-(−)-βγ-dihydroxybutyraldehyde [22] (kindly supplied by Dr. D. C. C. Smith), and smaller amounts of impurities having $R_F$ 0·25, 0·48, and 0·88.

*Oxidation of* D-(−)-βγ-*Dihydroxybutyraldehyde to* D-(−)-βγ-*Dihydroxybutyric Acid.*—The crude D-(−)-βγ-dihydroxybutyraldehyde obtained above was oxidised with bromine water in the presence of excess of barium carbonate in the usual way. Unchanged bromine and barium carbonate were removed, and the colourless solution was run through a column of Amberlite IR-120(H) resin (100 ml.). The acidic effluent was neutralised with silver carbonate, the precipitate of silver bromide filtered off, and silver removed from the filtrate by passing it through a column of Amberlite IR-120(H) resin (50 ml.). Concentration of the effluent gave a syrup which was examined by paper chromatography in solvent A. Spray *f* revealed a main component corresponding to βγ-dihydroxybutyric acid ($R_L$ 0·60), and smaller amounts of unknown acids having $R_L$ 0·32, 0·42, 0·76, and 0·91. Spray *e* revealed a single lactone spot corresponding to β-hydroxy-γ-butyrolactone ($R_L$ 1·04).

The acid syrup was transferred to four sheets of Whatman No. 3 MM paper (56 × 61 cm.) and the chromatogram developed in solvent A for 7 hr. Elution of the lactone bands from each paper gave a total neutral solution which was shown by titration in the usual way to contain 5·75 milliequiv. of lactone. Paper chromatography of this lactone as described gave a single spot corresponding to β-hydroxy-γ-butyrolactone ($R_L$ 1·03). A portion of the lactone was converted into brucine D-βγ-dihydroxybutyrate which crystallised readily from ethanol and had m. p. 178—179°, $[\alpha]_D^{20}$ −21·6° (Found: C, 62·8; H, 6·7; N, 5·5. Calc. for $C_{27}H_{34}O_8N_2$: C, 63·0; H, 6·6; N, 5·45%). Glattfeld *et al.*[10] gave m. p. 178°, $[\alpha]_D^{20}$ −29·42°, for this salt, and $[\alpha]_D^{20}$ −23·6° for the product obtained from maltose.

From a second portion of the lactone was obtained D-βγ-dihydroxy-*N*-phenylbutyrohydrazide, which after two crystallisations from ethyl acetate had m. p. 98—99° (Found: C, 57·1; H, 6·6; N, 13·1. Calc. for $C_{10}H_{14}N_2O_3$: C, 57·2; H, 6·7; N, 13·3%). Glattfeld *et al.*[10] record m. p. 102° for this phenylhydrazide.

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