385. *Mechanism of Saccharinic Acid Formation. Part II.*[1] *The αβ-Dicarbonyl Intermediate in Formation of* D-*Glucoisosaccharinic Acid.*

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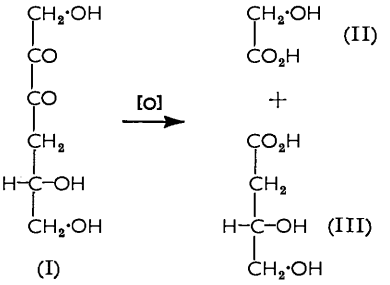
4-Deoxy-3-oxo-D-fructose has been isolated after treatment of maltose with sodium hydroxide, and characterised. This product gives a 90% yield of D-glucoisosaccharinic acid in lime-water and yields a complex mixture of acidic products in sodium hydroxide. From a comparison of acid yields it is concluded that all 4-*O*-substituted D-glucose derivatives, including cellulose and amylose, are degraded in alkali mainly to 4-deoxy-3-oxo-D-fructose, which may subsequently yield several different acidic products. Presence of calcium catalyses the benzilic acid rearrangement of 4-deoxy-3-oxo-D-fructose to D-glucoisosaccharinic acid and so reduces the relative yields of the other products. In dilute sodium hydroxide at 25°, considerable fragmentation to glycollic, βγ-dihydroxybutyric, and formic acid occurs.

IN the preceding paper[1] we reported the detection of a supposed intermediate in the degradation of maltose by dilute sodium hydroxide at 25°. By using similar conditions we have now isolated this intermediate and shown it to be 4-deoxy-3-oxo-D-fructose (I). αβ-Dicarbonyl compounds have often been postulated as intermediates in the alkaline degradation of carbohydrates to saccharinic acids, but have never previously been isolated from this type of reaction. The proof of structure is based on elemental analysis, preparation of a 2,4-dinitrophenylosazone in fair yield under very mild conditions, acetylation of the 2,4-dinitrophenylosazone, and oxidation by hydrogen peroxide.[2] Glycollic (II) and βγ-dihydroxybutyric acid (III) were identified as the major products of the oxidation, but



the quantitative results (Table 1) were complicated by overoxidation, particularly of glycollic acid.[3] The yield of βγ-dihydroxybutyric acid (82%) under optimum conditions confirms that the product is essentially 4-deoxy-3-oxo-D-fructose, but since it was isolated

TABLE 1. *Acids formed on oxidation of* 4-*deoxy*-3-*oxo*-D-*fructose* (2·5 *millimol.*) *with hydrogen peroxide.*

Duration of oxidation (hr.)	1	2·5	5
Total acid (milliequiv.)	4·84	4·99	4·32
Formic acid (milliequiv.)	0·33	0·70	0·96
Other volatile acids (milliequiv.)	0·21	0·45	0·72
Glycollic acid (milliequiv.)	1·22	1·35	2·64
βγ-Dihydroxybutyric (milliequiv.)	1·33 *	2·05	

* Lactone only; corresponding free acid not determined.

from an alkaline solution it is very probable that small amounts of isomers, resulting from tautomerisation, are present. Further, the product isolated will almost certainly exist

[1] Part I, Machell and Richards, preceding paper.
[2] Boeseken, *Rec. Trav. chim.*, 1911, **30**, 142.
[3] Hatcher and Holden, *Trans. Roy. Soc. Canada*, 1926, **20**, 407.

mainly as a mixture of cyclic forms and hydrates derived from (I), by analogy with the sugar osones.

Treatment of 4-deoxy-3-oxo-D-fructose with lime-water at 25° gave D-glucoisosaccharinic acid in 90% yield, and so confirmed the view that the former compound is an intermediate in formation of this acid. The high yield, however, does not necessarily mean that the product isolated has 90% purity, since the possible tautomeric contaminants would also be converted by way of (I) into D-glucoisosaccharinic acid in alkali. The acidic products resulting from degradation under various conditions of alkalinity and temperature were determined and are shown in Table 2. Comparison of these results with those from similar alkaline degradations of 4-*O*-methyl-D-glucose, maltose, amylose, and cellulose [1] shows considerable qualitative and quantitative similarity. We may therefore conclude that the alkaline degradation of all of the 4-*O*-substituted glucose derivatives so far studied proceeds mainly by elimination of the 4-*O*-substituent to yield the diketone (I), which subsequently is degraded by various mechanisms to give complex mixtures of acidic products.

TABLE 2. *Acids (% total acid equiv.) from 4-deoxy-3-oxo-D-fructose in alkali.*

Acid	0·04N-Lime-water; 25°	0·05N-Sodium hydroxide; 25°	0·5N-Sodium hydroxide; 100° †
Formic	1·7	19	20
Other volatile acids	0·7	1	4
D-Glucoisosaccharinic	90	23	33
Glycollic	×	19	6
βγ-Dihydroxybutyric ‡	×	26	16
Lactic	—	—	6

* Short-term (24 hr.) reaction. † Reaction carried out on neutral products from short-term treatment with 0·05N-sodium hydroxide (previous column). × Not determined. — Not detected. ‡ Lactone only; corresponding free acid not determined.

TABLE 3. *Rate of acid formation from 4-deoxy-3-oxo-D-fructose in 0·04N-lime-water at 25°.*

Time (hr.)	Acid formed (equiv./mole)	Time (hr.)	Acid formed (equiv./mole)	Time (hr.)	Acid formed (equiv./mole)	Time (hr.)	Acid formed (equiv./mole)
0·25	0·61	3	0·82	48	0·86	664	0·91
0·5	0·74	7	0·84	120	0·88	1628	0·94
1	0·78	24	0·85	288	0·89		

TABLE 4. *Rate of acid formation from 4-deoxy-3-oxo-D-fructose in 0·05N-sodium hydroxide at 25°.*

Time (hr.)	Acid formed (equiv./mole)	Time (hr.)	Acid formed (equiv./mole)	Time (hr.)	Acid formed (equiv./mole)	Time (hr.)	Acid formed (equiv./mole)
0·5	0·07	7·5	0·37	120	0·84	1152	1·18
2	0·17	24	0·67	288	0·90	1656	1·21
4·5	0·27	48	0·76	648	1·13		

A comparison of the rate of acid formation from 4-deoxy-3-oxo-D-fructose in lime-water (Table 3) and in 0·05N-sodium hydroxide (Table 4) at 25°, shows very much more rapid development of acidity in the former case. In view of the high yield of D-glucoisosaccharinic acid in lime-water, and of the known catalysis by calcium salts of the benzilic acid rearrangement of glyoxal,[4] we interpret these results as due to catalysis of the benzilic acid rearrangement of the diketone (I) to D-glucoisosaccharinic acid, possibly by $(CaOH)^+$. This is evidently the reason for the failure to detect this intermediate in previous studies of lime-water degradation of 4-*O*-substituted glucoses (see references cited earlier [1]).

The isolation of glycollic and βγ-dihydroxybutyric acid from alkaline degradation of 4-deoxy-3-oxo-D-fructose confirms the earlier suggestion [1] that in the alkaline degradation of 4-*O*-substituted glucoses these products arise by fragmentation of the diketone. The mechanism of the alkaline scission in αβ-dicarbonyl compounds is, however, unknown. In

[4] Machell and Richards, following paper.

the aromatic αβ-diketones the scission has usually been assumed to be hydrolytic, whereas for diacetyl it has been suggested that it may occur by a redox mechanism.[5] This type of reaction is reviewed elsewhere [6] and in the present case a decision between the two types of scission is not possible. The neutral products which would result from hydrolysis have not been detected. Glycollaldehyde would result from this type of scission and its 2,4-dinitrophenylhydrazone has therefore been prepared and attempts made to isolate the latter from treatment of the neutral products of alkaline degradation of the diketone (I). The results indicate that glycollaldehyde is not present in more than traces, but a preliminary investigation of the behaviour of glycollaldehyde in dilute sodium hydroxide at 25° showed rapid disappearance so that it may still be a primary product of alkaline degradation of the diketone (I).

Lactic acid was not detected as a product of short-term treatment of the diketone (I) with dilute sodium hydroxide at 25°; but, when the neutral products of such a reaction were isolated and treated with sodium hydroxide at 100°, a considerable amount of lactic acid was obtained (Table 1). These experiments suggest that the lactic acid is derived from a primary neutral product of alkaline degradation of the diketone, but they are not in accordance with the earlier suggestion [7] of the intermediate formation of a ketohexose, since such an intermediate would yield lactic acid comparatively rapidly.

Considerable quantities of formic acid were obtained on treatment of the diketone with sodium hydroxide. The source of this acid is not known, but it may be associated with the formation of the 3-deoxy-D-pentonic acids which have repeatedly been detected by paper chromatography.

Experimental

The following solvents and sprays were used for chromatography on Whatman No. 1 paper at 25°: solvents; A, butan-1-ol–pyridine–water (6 : 4 : 3); B,[8] ethyl acetate–acetic acid–water (10 : 1·3 : 1); C, butan-1-ol–ethanol–water (4 : 1·1 : 1·9). Sprays: *a*,[9] silver nitrate–sodium hydroxide; *b*, a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid; *c*,[10] B.D.H. 4·5 indicator; *d*,[11] hydroxylamine–ferric chloride; *e*,[12] naphtharesorcinol–2N-hydrochloric acid.

Total acids in solutions containing lactonisable acids were determined by adding a two-fold excess of alkali, storing the mixture for 30 min. at room temperature, and titrating it with acid to pH 9. Free acids in the same solutions were titrated directly to a transient end-point with phenolphthalein.

Degradation of Maltose in 0·05N-Sodium Hydroxide at 25°.—(a) *Reaction rate.* Maltose monohydrate (4·49 g.) was dissolved in an equivalent amount (250 ml.) of 0·05N-sodium hydroxide in the absence of oxygen, and the solution kept at 25°. Aliquot portions (20 ml.) were withdrawn at intervals, added to excess of 0·05N-hydrochloric acid (20 ml.), and titrated immediately with 0·01N-sodium hydroxide. The amount of acid formed was: 0·002 (1 hr.), 0·006 (2 hr.), 0·009 (3 hr.), 0·012 (4 hr.), 0·016 (5 hr.), 0·018 (6 hr.), and 0·022 (7 hr.) equiv./mole.

Further samples (1 ml.) were withdrawn at 1 hr. intervals, treated with Amberlite IR-120(H) resin (0·5 ml.), and the resulting solutions examined by paper chromatography in solvent A. Spray *a* revealed maltose, R_F 0·24, glucose, R_F 0·37, and a component, R_F 0·74, which also reacted rapidly with spray *b*. Visual estimation indicated that the concentration of the latter component reached a maximum after reaction for 5 hr.

(b) *Isolation of neutral products.* Maltose monohydrate (81 g.) was treated with 0·05N-sodium hydroxide (4·5 l.) as described under (a). After 5 hr., the pH of the solution was quickly reduced to 8 by the step-wise addition of Amberlite IR-120(H) resin (*ca.* 135 ml.) with stirring, the resin filtered off, and the filtrate evaporated under reduced pressure to *ca.* 250 ml.

[5] von Euler and Hasselquist, *Arkiv Kemi*, 1949, **1**, 325.
[6] Machell, *J.*, 1960, 683.
[7] Richards and Sephton, *J.*, 1957, 4492.
[8] Richtzenhain and Moilanen, *Acta Chem. Scand.*, 1954, **8**, 704.
[9] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[10] Nair and Muthe, *Naturwiss.*, 1956, **43**, 106.
[11] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[12] Hough, Jones, and Wadman, *J.*, 1950, 1702.

The concentrate was then deionised by adding, with stirring, a mixture of the Amberlite resins IR-120(H) (20 ml.) and IRA-400 (carbonate) (40 ml.), the pH of the solution being kept above 4·5 by a suitable rate of addition. The final conductivity of the solution was <10 μmho. After removal of the resins by filtration, the solution was evaporated to a syrup under reduced pressure, and the syrup dried to constant weight (77 g.) over phosphoric oxide *in vacuo*. Paper chromatography of the syrup as in (a) indicated the presence of small amounts of the component of R_F 0·74.

Attempts were made to extract this carbonyl compound from the syrup by using solvent mixtures, *e.g.*, methanol–ether and methanol–acetone in various proportions. However, even in the most favourable case, the extent of enrichment was more than offset by the small amount of extract obtained. The whole of the syrup was then transferred to a large cellulose column (75 × 4·5 cm.) and eluted with 98% ethanol. Paper chromatography of the eluate fractions indicated that virtually all the desired carbonyl compound was contained in the initial 1·5 l. of eluate. However, traces of glucose and an unknown compound, of R_F 0·80 (solvent A), were also present. Evaporation of the eluate, followed by drying of the residue over phosphoric oxide *in vacuo*, yielded a syrup (0·7 g.).

Three batches of impure carbonyl compound were isolated in this way, combined, and transferred to a smaller cellulose column (60 × 3 cm.). Elution with ethyl methyl ketone–water azeotrope afforded a component, R_F 0·74 (solvent A), which was free from impurities. The dried residue of carbonyl compound was taken up in water (20 ml.) and stored at 0° overnight, and then a small amount of white solid was filtered off. The filtrate was then evaporated under reduced pressure and, after drying over phosphoric oxide at 50°/0·1 mm., there was obtained, as a yellow-green hygroscopic syrup, 4-*deoxy*-3-*oxo*-D-*fructose* (1·35 g.), $[\alpha]_D^{22}$ −24·1° (*c* 3·32 in H_2O), $\lambda_{max.}$ 2780 Å (ε 80) (Found: C, 44·3; H, 6·65. $C_6H_{10}O_5$ requires C, 44·4; H, 6·2%). A methanolic solution of this compound gave no colour with a solution of ferric chloride in the same solvent; after the addition of pyridine, however, a wine-red colour resulted (cf. 4-hydroxypentane-2,3-dione).[13]

Preparation of Derivatives.—Crude 4-deoxy-3-oxo-D-fructose (0·55 g.) was added to a saturated solution (800 ml.) of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid at 20°. An orange-red precipitate was formed immediately; after 1 hr., this was filtered off and extracted with boiling alcohol to remove the monohydrazone impurity. The residue (0·78 g.), crystallised twice from anisole, afforded 4-*deoxy*-3-*oxo*-D-*fructose bis*-2,4-*dinitrophenylhydrazone*, m. p. 256° (Found: C, 41·6; H, 3·5; N, 21·6. $C_{18}H_{18}O_{11}N_8$ requires C,41·4; H, 3·45; N, 21·5%).

A portion of this hydrazone (0·2 g.) was dissolved in dry pyridine (1 ml.), and acetic anhydride (0·7 ml.) was added. After 24 hr. at room temperature, the product was poured into water, and the precipitate collected. After crystallisation from ethyl acetate–ethanol and recrystallisation from ethyl acetate alone, the *triacetate* had m. p. 200—201° (Found: C, 44·1; H, 3·8; N, 17·0. $C_{24}H_{24}O_{14}N_8$ requires C, 44·4; H, 3·7; N, 17·3%).

Oxidation of 4-*Deoxy*-3-*oxo*-D-*fructose with Hydrogen Peroxide.*—(a) *Identification of products.* 4-Deoxy-3-oxo-D-fructose (0·4 g.) in water (4 ml.) was treated with 30% hydrogen peroxide (4 ml.) at 100° for 2·5 hr. Unchanged peroxide was then decomposed by adding a trace of catalase to the diluted acidic product, and the acids were separated, by distillation of the aqueous solution at 50°/12 mm., into volatile and non-volatile fractions. The presence of formic acid in the former was established by qualitative tests, while the same fraction gave a precipitate with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid. Crystallisation of this hydrazone precipitate from glacial acetic acid afforded a dark red solid (*ca.* 20 mg.), m. p. 270° (decomp.), which was not examined further.

Paper chromatography of the solution of non-volatile acids with solvent B and sprays *c* and *d* revealed components corresponding to βγ-dihydroxybutyric acid (R_L 0·60), glycollic acid (R_L 0·76), and β-hydroxy-γ-butyrolactone (R_L 1·03). Traces of two unknown acids, R_L 0·65 and 0·82 severally, were also present (subscript L refers to lactic acid). A portion of the acid solution was transferred to a Whatman No. 3 MM paper (56 × 61 cm.) and developed in solvent B. The components corresponding to (i) glycollic acid and (ii) β-hydroxy-γ-butyrolactone were separately eluted with water. From eluate (i) was prepared 4-bromophenacyl glycollate, having m. p. and mixed m. p. 141—143° after two crystallisations from benzene. Eluate (ii) was treated with excess of brucine at 100°, and the brucine βγ-dihydroxybutyrate produced crystallised from ethanol (m. p. and mixed m. p. 179—180°).

[13] Hesse and Stahl, *Chem. Ber.*, 1956, **89**, 2414.

(b) *Rate of formation of acidic products.* With the amounts indicated under (a), three further oxidation experiments were carried out for the following reaction times: (i) 1 hr., (ii) 2·5 hr., (iii) 5 hr. In each case, the proportions of volatile and non-volatile acids, and the formic acid in the former fraction, were determined. Paper chromatography of the non-volatile acids as in (a) gave similar results to those reported therein, except that the product from (i) contained a larger amount of the unknown acid, R_L 0·65. In case (ii), a portion of the non-volatile acid was separated on a Whatman No. 3 MM paper as described, and the proportions of glycollic and βγ-dihydroxybutyric acid + its lactone were determined as described elsewhere.[1] In case (i), the presence of a significant amount of the acid, R_L 0·65, prevented determination of the free βγ-dihydroxybutyric acid, and only the yield of the corresponding lactone is reported. Owing to the high proportion of volatile acids obtained in case (iii), analysis of the non-volatile fraction was not undertaken. Results are recorded in Table 1.

Action of Lime-water on 4-Deoxy-3-oxo-D-fructose at 25°.—(a) *Rate of acid formation.* A solution of 4-deoxy-3-oxo-D-fructose (0·196 g.) in water (5 ml.) was diluted to 100 ml. with 0·042N-lime-water, then kept at 25° ± 0·1° in the absence of oxygen, the initial deep yellow colour being gradually discharged. It was not possible to measure the rate of acid formation by back-titration as described earlier, since it was too fast for a reliable " blank " to be determined for 0 hr. Consequently, aliquot portions (5 ml.) of solution were withdrawn and stirred with Amberlite IR-120(H) resin (0·5 ml.) for 5 min., the resin was quickly filtered off, and the acid in the filtrate titrated immediately with 0·01N-sodium hydroxide. Results are shown in Table 3.

(b) *Isolation of products.* 4-Deoxy-3-oxo-D-fructose (1·34 g.) was added to oxygen-free 0·042N-lime-water (1 l.) at 25°. After 24 hr. at this temperature, the solution was neutralised by carbon dioxide, and then evaporated to *ca.* 100 ml. under reduced pressure. The filtered concentrate was passed through a column of Amberlite IR-120(H) resin (30 ml.) directly into a stirred suspension of De-Acidite FF resin (200—400 mesh; low cross-linked) (30 g., air-dried) in the carbonate form. After 24 hours' stirring the resin was filtered off and washed with water, and the combined filtrate and washings were evaporated under reduced pressure. Further drying over phosphoric oxide *in vacuo* afforded a syrup (45 mg.) which was examined by paper chromatography in solvent C. Spray *b* revealed components of R_F 0·29, 0·38, 0·49 (unchanged starting material), 0·59, 0·67, and 0·75, while additional components of R_F 0·09 and 0·14 were developed by spray *a*. This complex mixture was not investigated further.

Acids were eluted from the De-Acidite resin by N-ammonium carbonate (600 ml.) in 4 hr., the eluate evaporated to dryness under reduced pressure, and the residue finally heated at 70°/15 min. to decompose the excess of eluent. The residue of ammonium salts was taken up in water (25 ml.), the solution treated with Amberlite IR-120(H) resin (15 ml.) in a column, and the acidic effluent diluted to 100 ml. Titration of an aliquot portion of this solution established the presence of 7·50 milliequiv. of total acid. An initial separation of the acids in this solution by methods previously described gave the following results on an equivalent basis: formic acid, 1·7%; other volatile acids, 0·7%; non-volatile acids, 97·6%.

Paper chromatography of the non-volatile acids in solvent B with sprays *c* and *d* revealed components corresponding to D-glucoisosaccharinic acid, R_L *ca.* 0·15, and D-glucoisosaccharinolactone, R_L 0·58. With very heavy loading, traces of material corresponding to glycollic acid, R_L 0·75, and β-hydroxy-γ-butyrolactone, R_L 1·04, were also detected. A portion (5 milliequiv.) of the non-volatile acid was heated with excess of calcium hydroxide at 80° for 10 min. Unused hydroxide was then filtered off, and the filtrate neutralised with carbon dioxide, and re-heated at 80° for 10 min. After filtration from calcium carbonate, the solution of calcium salts was concentrated to 20 ml. under reduced pressure, and then stored at 2° for several days. The crystals of calcium α-D-glucoisosaccharinate (0·13 g.) obtained were collected, then dissolved in water, and treated with Amberlite IR-120(H) resin (5 ml.) in a column. Evaporation of the effluent to dryness under reduced pressure, followed by drying over phosphoric oxide *in vacuo* to induce lactonisation, yielded a syrup (0·11 g.). This was extracted with boiling ethyl acetate, and from the extract, after recrystallisation from ethyl acetate–light petroleum, was obtained α-D-glucoisosaccharinolactone, m. p. and mixed m. p. 90—92°.

A further portion of the solution of non-volatile acids was transferred to a Whatman No. 3 MM paper, and the proportion of D-glucoisosaccharinic acid + lactone determined as previously described, and found to be 90% of the total acid produced in the original alkaline treatment.

Action of Sodium Hydroxide on 4-Deoxy-3-oxo-D-fructose at 25°.—(a) *Rate of acid formation.*

A solution of 4-deoxy-3-oxo-D-fructose (0·205 g.) in water (*ca.* 5 ml.) was diluted to 100 ml. with 0·05N-sodium hydroxide, then kept at 25° with oxygen absent, the original pale yellow colour changing to brown. Aliquot portions (5 ml.) of the solution were at intervals added to 0·05N-hydrochloric acid (5 ml.), the excess of acid being titrated immediately with 0·01N-sodium hydroxide. As a check, several similar portions of solution were examined by the resin method detailed above; satisfactory agreement was obtained. Results are recorded in Table 4.

(b) *Isolation of products from short-term reaction.* 4-Deoxy-3-oxo-D-fructose (1·35 g.) was treated with 0·05N-sodium hydroxide (250 ml.) under oxygen-free conditions at 25°. After 24 hr., the neutral (see d below) and acidic (5·50 milliequiv.) products were separated as described for the lime-water reaction, and the acidic products then separated further into volatile and non-volatile fractions. The volatile acid readily yielded 4-bromophenacyl formate, which, crystallised from aqueous ethanol, had m. p. and mixed m. p. 138—139°. The proportion of formic acid in the volatile fraction was then determined. Paper chromatography of the non-volatile acid fraction in solvent B with sprays *c* and *d* revealed components corresponding to D-glucoisosaccharinic acid, R_L 0·17, and the related lactone, R_L 0·58, βγ-dihydroxybutyric acid, R_L 0·60, glycollic acid, R_L 0·76, and β-hydroxy-γ-butyrolactone, R_L 1·04. There was also a trace of an unknown acid, R_L *ca.* 0·55, and small amounts of two lactones, R_L 0·69 and 0·78 respectively, which correspond to the two 3-deoxy-D-pentonolactones. Even with very heavy loading of the chromatogram, lactic acid (R_L 1·00) was not detected.

A portion of the non-volatile acid solution was separated on a Whatman No. 3 MM paper, and the components corresponding to β-hydroxy-γ-butyrolactone eluted with water. From the eluate was prepared brucine βγ-dihydroxybutyrate, m. p. and mixed m. p. 180°. From a second portion of the solution of non-volatile acids, the proportions of the acids were determined by chromatography on Whatman No. 3 MM paper. The results obtained under this heading are now summarised, the proportions of the acids produced being: formic, 19%; other volatile acids, 1%; D-glucoisosaccharinic, 23%; glycollic, 19%; and β-hydroxy-γ-butyrolactone, 26%. It was not possible to determine the free βγ-dihydroxybutyric acid owing to the complexity of the mixture.

(c) *Long-term reaction.* 4-Deoxy-3-oxo-D-fructose (0·021 g.) was treated with 0·05N-sodium hydroxide at 25° as described under (b), but the reaction time was increased to 1500 hr. Paper chromatography of the non-volatile acids then produced revealed components corresponding to those obtained in the 24 hr. reaction, but, in addition, material corresponding to lactic acid, R_L 1·00, was readily detected.

(d) *Neutral products.* The neutral products from (b) were examined by paper chromatography in solvent A; spray *b* revealed components of R_F 0·56, 0·63, and 0·70. Application of spray *e* produced a single vivid blue spot, R_F 0·56; with the same spray, βγ-dihydroxybutyraldehyde (kindly provided by Dr. D. C. C. Smith) gave a purple spot, R_F 0·68, and glycollaldehyde a vivid blue spot, R_F 0·73.

A portion (0·36 g.) of the neutral products was treated with a solution of 2,4-dinitrophenylhydrazine (1·0 g.) in 0·5N-hydrochloric acid (500 ml.). Slow precipitation commenced after 5 min., but the mixture of products was not resolved.

Action of Sodium Hydroxide on the Neutral Products at 100°.—A second batch of neutral products was obtained by repetition of the short-term sodium hydroxide reaction described above. This material was treated with 0·5N-sodium hydroxide for 1 hr. at 100° in the absence of oxygen, and, after cooling, the dark brown product was separated into acidic (3·16 milliequiv.) and neutral fractions, the latter being rejected. Paper chromatography of the acids revealed components corresponding to those recorded for the sodium hydroxide reactions already described, including lactic acid, R_L 1·00. Analysis of the acidic solution afforded the following results for the proportions of acids present: formic, 20%; other volatile, 4%; D-glucoisosaccharinic, 33%; glycollic, 6%; and lactic, 6%. The proportion of β-hydroxy-γ-butyrolactone (free acid not determined) was 16%.

Reaction of Glycollaldehyde with 2,4-Dinitrophenylhydrazine.—Glycollaldehyde (0·068 g.) was treated with a solution of 2,4-dinitrophenylhydrazine (0·2 g.) in 0·5N-hydrochloric acid (150 ml.). The flocculent yellow precipitate was filtered off after 15 min., and after crystallisation first from hot water, and then from ethanol, gave orange needles (0·160 g.) of *glycollaldehyde 2,4-dinitrophenylhydrazone*, m. p. 162—163° (Found: C, 39·7; H, 3·3; N, 23·4. $C_8H_8O_5N_4$ requires C, 40·0; H, 3·3; N, 23·3%).

Action of Sodium Hydroxide on Glycollaldehyde at 25°.—Glycollaldehyde (0·076 g.) was

dissolved in 0·05N-sodium hydroxide (250 ml.) at 25° in the absence of oxygen. Aliquot portions (25 ml.) of this solution were withdrawn at intervals, and deionised with a mixture of Amberlite resins IR-120(H) (2·5 ml.) and IRA-400(carbonate) (5 ml.). Paper chromatography of the resulting solutions in solvent A with spray *a* indicated that the glycollaldehyde (R_F 0·73) had reacted rapidly with the alkali, and after 4 hr. appeared to have been almost completely consumed. The solution then remaining was complex, containing components with the following R_F values: 0·28, 0·36, 0·44, 0·56, and 0·60, the last two also reacting with spray *b*.

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