238. *The Alkaline Degradation of Polysaccharides. Part IV.* Monosaccharide Analogues of Periodate Oxycellulose.*

By D. O'Meara and G. N. Richards.

A study of the products of alkaline degradation of certain periodate-oxidised monosaccharides suggests that the degradation occurs predominantly by scission of the carbon–oxygen linkage in the β-position with respect to the aldehyde groups. Subsequent rearrangements of the products probably yield a molecule of glycollic acid from each scission of this type. In certain circumstances an intramolecular Cannizzaro rearrangement can occur in the periodate-oxidation product. The analogy with periodate oxycellulose is discussed.

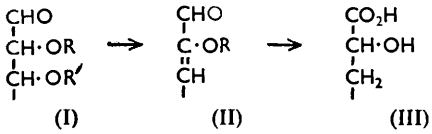
Oxidised celluloses which contain carbonyl groups are known to undergo chain scission by alkali and this effect has been interpreted by various workers in terms of alkaline hydrolysis of glucosides [1] and acetals,[3] keten acetals,[2] and carbonate esters,[3b] and of β-alkoxycarbonyl elimination.[4] In each case similar arguments could equally well be applied to oxidised amylose.[5] However, the general effect of alkaline elimination of an alkoxyl group from the β-position to a carbonyl group has been proved for simpler compounds such as the *O*-substituted hexoses.[6] In order to extend such an analogy to an oxidised polysaccharide it is necessary to introduce a single type of oxidised group into a chemically pure polysaccharide and a specific oxidant is therefore required. Such a case is periodate oxycellulose. As a preliminary, and in order to develop techniques, we have first considered analogues of low molecular weight.

For periodate oxycellulose we assume that alkaline degradation will not appreciably affect secondary cyclic systems resulting from internal acetal formation by the aldehyde

* Part III, preceding paper.

[1] Davidson, *J. Text. Inst.*, 1938, **29**, T 195.
[2] Pacsu, *Text. Res. J.*, 1945, **15**, 354.
[3] (*a*) Head, *J. Text. Inst.*, 1947, **38**, T 389; (*b*) Kaverzneva, Ivanov, and Salova, *Bull. Acad. Sci.* U.S.S.R., 1952, 681.
[4] Haskins and Hogsed, *J. Org. Chem.*, 1950, **15**, 1264; Corbett, Kenner, and Richards, *Chem. and Ind.*, 1953, 154; Kenner, *ibid.*, 1955, 727.
[5] Cf. Anderson, Greenwood, and Hirst, *J.*, 1955, 225; Hofreiter, Alexander, and Wolff, *Analyt. Chem.*, 1955, **27**, 1930.
[6] Kenner and Richards, *J.*, 1957, 2916, and earlier work.

groups.[7] The structure of an oxidised unit (XIV) is therefore such that, although β-alkoxycarbonyl groupings are present (*a*), the α-hydroxycarbonyl grouping is absent. Now work on 2 : 3-di-*O*-methyl-D-glucose [8] indicates that the normal alkaline degradation of a β-alkoxycarbonyl system (I) will only proceed to completion when the product (II) can undergo further reaction, *e.g.*, to produce a saccharinic acid (III). The latter reaction is evidently dependent on the nature of the group R and probably only occurs readily, as



(I) (II) (III)

shown, when R = H. In such cases (*i.e.*, β-alkoxycarbonyl-α-hydroxy systems), the indications are that the reaction sequence (I) ⟶ (III), particularly in the presence of calcium ions, proceeds almost to completion. In the case of 2 : 3-di-*O*-methyl-D-glucose however, where R = R′ = Me, the product (II) was evidently stable in dilute alkali at room temperature and the acid (III) was not produced under these conditions.

Suitable monosaccharide analogues of periodate oxycellulose must therefore contain substituents which replace the groups adjacent to the oxidised unit in (XIV). We chose the periodate-oxidation product (IV) of methyl 4 : 6-*O*-benzylidene-α-D-glucoside, and the product (V) of its partial acidic hydrolysis. α-Anomers were chosen for convenience of preparation and this configuration is not expected to influence comparison with periodate oxycellulose.

Additional acetal and hemiacetal cyclic systems which are possibly present in the material (IV) and almost certainly present in the product (V) are assumed not to have any marked influence on the alkaline degradation. The relative stability of the latter towards acidic hydrolysis suggests, however, possible formation of a six-membered ring,[7] and its slow reaction with sodium metaperiodate probably indicates ring closure between $C_{(2)}$ and $C_{(4)}$ to give the dioxan (VI), or possibly a fused-ring system resulting from further acetal formation by the remaining free aldehyde group.

The benzylidene derivative (IV), which carries a cyclic acetal substituent in the α-carbonyl position, was treated with lime-water at room temperature. Although only sparingly soluble in water, it dissolved much more readily in lime-water and was reprecipitated unchanged if the solution was immediately saturated with carbon dioxide (this may indicate salt or complex formation). After 30 min. at room temperature the lime-water solution yielded glycollic acid (IX) and 4-formyl-2-phenyl-2*H*, 6*H*-1 : 3-dioxin (VII) in approximately equal amounts, together with unchanged material (IV) and products arising from a Cannizzaro reaction. Identification of the dioxin derivative (VII) is based on its elemental analysis, the yield of benzaldehyde on acidic hydrolysis, and on the fact that after acidic hydrolysis and treatment with lime-water paper-chromatographic evidence of αγ-dihydroxybutyric acid (X) was obtained.

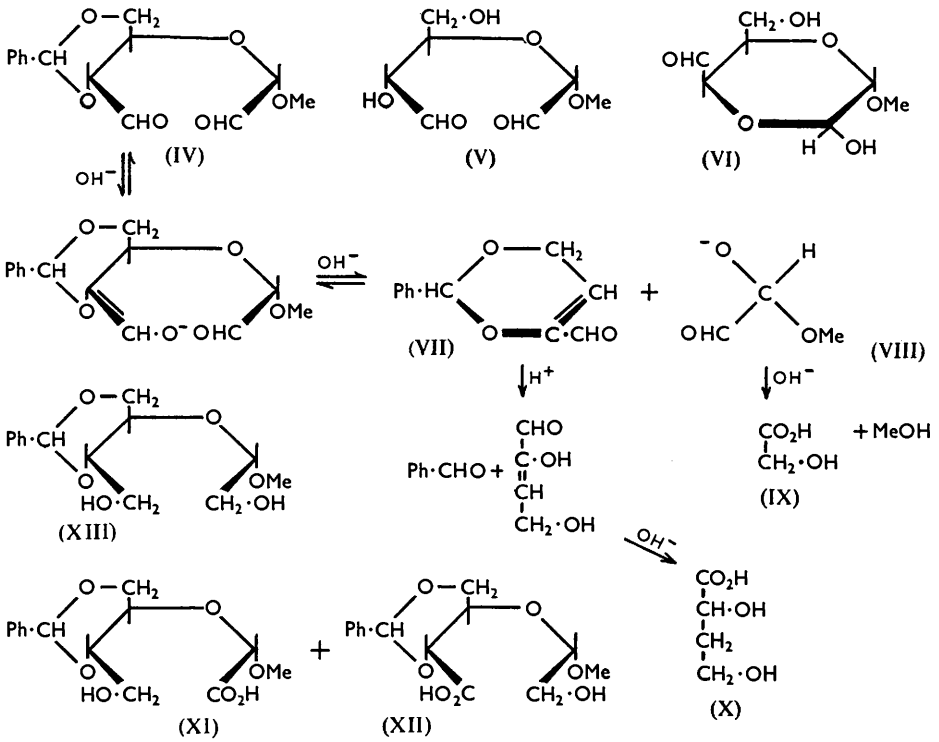
From this degradation we also isolated a mixture of the acids (XI) and (XII) (approx. ratio 3 : 1) arising from an intramolecular Cannizzaro reaction of the starting material (IV). This reaction apparently accounted for about 20% of the starting material decomposed for both short and long treatments. No neutral product (XIII) could be detected, so it was concluded that intermolecular Cannizzaro reactions were not important. The dioxin derivative (VII) decomposed slowly in lime-water at room temperature, liberating benzaldehyde and a complex mixture of acidic products, and depositing an insoluble resin; this degradation was evidently complex and was not investigated.

Degradation of dialdehyde (V) by lime-water at 25° resulted in the rapid formation of

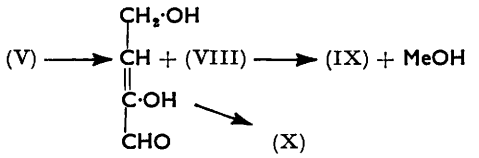
[7] Cf. Cadotte, Dutton, Goldstein, Lewis, Smith, and van Cleve, *J. Amer. Chem. Soc.*, 1957, **79**, 691; Richards, *J.*, 1957, 3222.

[8] Kenner and Richards, *J.*, 1956, 2921.

glycollic (IX) and αγ-dihydroxybutyric (X) acids, which were shown by quantitative paper chromatography to be present in approximately equivalent amount and to correspond to 72% degradation of the aldehyde (V) after 24 hr. Both acids were identified as crystalline derivatives. Volatile acids (10% of total acidity) produced simultaneously consisted mostly of formic acid, and unidentified neutral compounds were also formed



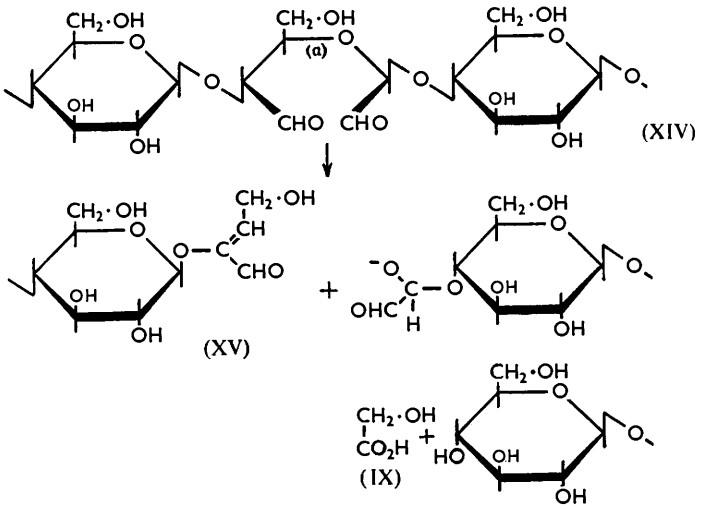
(*ca.* 15%, possibly including unchanged V) under these conditions. Further slow formation of small amounts of acid in the lime-water solution was not studied further. No conclusive evidence was obtained of a Cannizzaro reaction but qualitative paper chromatography suggested that it may occur to a very small extent.



Each of the products of the reactions described above is in accordance with the reaction mechanisms shown and it is therefore probable that the same type of alkaline degradation will apply also in periodate oxycellulose. This implies that the alkaline degradation of periodate oxycellulose, such as was observed by Davidson,[1] ultimately results from scission of the C–O bond (a) of the oxidised unit, as proposed by Haskins and Hogsed [4] and by Corbett and Kenner.[9] Thus in the case of an isolated oxidised unit the scission may be represented as illustrated.

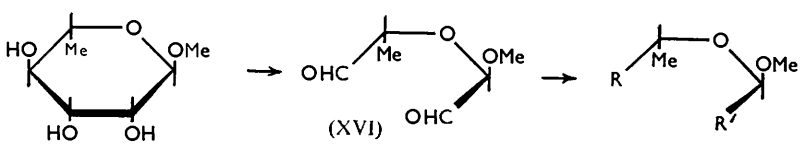
[9] Corbett and Kenner, *J.*, 1953, 2245.

Further, the equivalence of the yields of glycollic and αγ-dihydroxybutyric acids from alkaline degradation of the aldehyde (V) is very significant. Earlier experiments on the alkaline degradation of 3-*O*-methyl-D-glucose [10] indicated that the rearrangements which result in formation of D-gluco*meta*saccharinic acid, proceed almost quantitatively in lime-water at room temperature, and this system is analogous to the sequence of reactions



which result in formation of αγ-dihydroxybutyric acid (X) from the aldehyde (V). Hence the observation that glycollic and αγ-dihydroxybutyric acid were formed in equivalent amount by action of lime-water may be interpreted as an indication that the glyoxal derivative (VIII) undergoes complete degradation to glycollic acid (IX) as shown above. This was supported by identification of methanol. It follows that one molecule of glycollic acid should be liberated for each chain scission under these conditions.

The extent of subsequent alkaline rearrangement of an intermediate of type (XV) is however much less certain. Although Haskins and Hogsed [4] have suggested that an intermediate of this type will undergo simultaneous hydrolysis and hydration with formation of a tetrose, this seems unlikely. Thus for example, in the case of the benzylidene derivative (IV), where the glucosyl acetal linkage is replaced by a cyclic acetal grouping, the relevant intermediate (VII) was comparatively stable to lime-water, but underwent a slow and apparently complex degradation at room temperature, with liberation of benzaldehyde. The possibility of degradation of the intermediate (XV) from the oxycellulose is dependent on scission of the glycosyl linkage in (XV) and this will be further investigated.



R = CH_2·OH, R′ = CO_2H, or *vice versa*.

It is probable that not every oxidised unit in periodate oxycellulose will undergo scission in alkali: Cannizzaro rearrangement may intervene, and would yield an acidic polysaccharide. The Cannizzaro rearrangement has previously been observed [11,2] with

[10] Kenner and Richards, *J.*, 1954, 278.
[11] Fry, Wilson, and Hudson, *J. Amer. Chem. Soc.*, 1942, **64**, 872.

periodate-oxidation products such as (XVI), which do not contain a β-alkoxycarbonyl grouping. The results were therefore not complicated by the simultaneous occurrence of the scission reaction and it is probable that some of the periodate-oxidation products whose alkaline degradation was studied by Head [3] also underwent the Cannizzaro rearrangement for similar reasons.

Cannizzaro rearrangements have previously been postulated [5] to explain the acids produced by action of alkali on periodate-oxidised starches, but it is probable that most of the acidity observed was due to simple acids arising by scission. The apparently greater importance of the intramolecular Cannizzaro reaction for the benzylidene derivative (IV) than for its hydrolysis product (V) may be due to steric factors or to a slower scission as a consequence of the alkali-stability of the dioxin intermediate (VII). Experimental difficulties involved in studying the rate of alkaline degradation of the compound (IV) have so far prevented further investigation of this.

The modified Barry degradation,[12] which utilises *cyclo*hexylamine instead of phenylhydrazine acetate, almost certainly involves the type of alkaline degradation indicated above, which is particularly favoured by separation of an insoluble derivative of the glyoxal intermediate.

Experimental

The following solvents and sprays were used in paper chromatography. Solvent *a*, butan-1-ol–pyridine–benzene–water (4 : 2 : 1 : 1); solvent *b*,[13] ethyl acetate–acetic acid–water (10 : 1 : 3 : 1); solvent *c*,[14] ethyl acetate–acetic acid–water (3 : 1 : 1); solvent *d*, butan-1-ol–ethanol–water (4 : 1·1 : 1·9); spray *a*,[16] B.D.H. " 4·5 " indicator; spray *b*,[17] sodium periodate–permangate; spray *c*, 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid; spray *d*,[18] *p*-anisidine; spray *e*,[19] silver nitrate–sodium hydroxide; spray *f*,[20] hydroxylamine–ferric chloride.

Acid Determinations.—(a) *Total acids.* All acid yields were determined by back-titration after treatment at room temperature for 30 min. with a four-fold excess of 0·025N-sodium hydroxide, the final concentration of alkali being not less than 0·01N.

(b) *Volatile acids.* Volatile acids and the proportion of formic acid were determined as described earlier.[21]

(c) *Glycollic acid.* After separation by paper chromatography glycollic acid in aqueous solution was determined spectrophotometrically by Calkins's method.[22]

2 : 4-O-*Benzylidene*-3-O-(D-1-*methoxy*-2-*oxoethyl*)-D-*erythrose*.—This compound, m. p. 142—144°, was prepared by oxidation with sodium metaperiodate of methyl 4 : 6-*O*-benzylidene-α-D-glucoside.[23]

Alkaline Degradation.—(a) *Qualitative.* A suspension of the benzylidene derivative (12·0 g.) was stirred with oxygen-free 0·04N-calcium hydroxide (3950 ml.) at 20°. After *ca.* 1 hr. the smell of benzaldehyde became apparent and a brown resin separated.[24] After 16 hr. the mixture was filtered from resin and unchanged material (total wt. 3·92 g.), and excess of carbon dioxide was added to the filtrate which was then extracted with ether (3 × 500 ml.). The extracts were evaporated leaving, after removal of benzaldehyde by steam-distillation [2 : 4-dinitrophenylhydrazone (0·4 g.), m. p. 228—232°], a syrupy residue (0·40 g.), R_F 0·88 in solvent *d* (sprays *b*, *c*, *e*). This product, 4-*formyl*-2-*phenyl*-2H,6H-1 : 3-*dioxin*, recrystallised from ether

[12] Barry and Mitchell, *J.*, 1953, 3610.
[13] Moilanen and Richtzenhain, *Acta. Chem. Scand.*, 1954, **8**, 704.
[14] Loffler and Reichl, *Mikrochim. Acta.*, 1953, 79.
[15] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.
[16] Nair and Muthe, *Naturwiss.*, 1956, **43**, 106.
[17] Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.
[18] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[19] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[20] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[21] Richards and Sephton, *J.*, 1957, 4492.
[22] Calkins, *Analyt. Chem.*, 1943, **15**, 762.
[23] Rowen, Forziati, and Reeves, *J. Amer. Chem. Soc.*, 1951, **73**, 4484.
[24] Cf. Kenner and Richards, *J.*, 1955, 1709.

as needles, m. p. 70—71° (Found: C, 69·2; H, 5·4; Ph·CHO, 53·8. $C_{11}H_{10}O_3$ requires C, 69·5; H, 5·3; Ph·CHO, 55·6%). The product slowly decomposed at room temperature.

The aqueous residue after concentration to *ca.* 800 ml. was filtered from calcium carbonate and, after further concentration to 100 ml., the precipitated calcium glycollate tetrahydrate (3·66 g.) [Found: H_2O, 27·4. Calc. for $(C_2H_3O_3)_2Ca,4H_2O$: H_2O, 27·5%] was separated and characterised as its 4-bromophenacyl ester, m. p. and mixed m. p. 138—141°. Evaporation to dryness yielded a further quantity of calcium salts (3·90 g.) of which part (1·9 g.) dissolved in hot absolute alcohol (150 ml.). Treatment with Amberlite resin IRC-50 followed by paper chromatography revealed glycollic acid (R_F 0·51, solvent *c*) and a component R_F 0·82 (solvent *c*; sprays *a*, *c*, *d*). Treatment of this alcohol-soluble material with a mixture of Amberlite resins IR-120(H) and IR-4B(OH) removed the material completely from solution. Hydrolysis in 0·1N-hydrochloric acid at 60° for 1 hr. gave a mixture containing benzaldehyde, glyoxylic acid, glycollaldehyde, and erythritol, which were each identified as described below. A component corresponding to D-erythronolactone was disclosed by paper chromatography (R_F 0·39; solvent *b*, sprays *e*, *f*).

(b) *Quantitative.* The finely powdered erythrose derivative (9·48 g., 30 mmole) was stirred with oxygen-free 0·041N-calcium hydroxide (2·5 l.) at room temperature. After 20 min. the material had almost completely dissolved and excess of carbon dioxide was added. The unchanged material (5·1 g., 16·1 mmoles) which then separated was removed and the filtrate concentrated to 900 ml. The precipitated calcium carbonate together with some more unchanged material (0·60 g.; 1·9 mmoles) was filtered off and the filtrate extracted continuously with ether for 24 hr. The ethereal extract was concentrated to 30 ml., filtered from unchanged material (0·12 g., 0·33 mmoles) and on evaporation to dryness yielded the crude dioxin (1·453 g., 7·65 mmole), m. p. 58—60° rising to 70—71° after one recrystallisation from ether.

An aliquot portion (5 ml.) of the ether-extracted aqueous solution (total vol. 875 ml.) was stirred with Amberlite resin IR-120(H) (2·0 ml.) for 10 min., then filtered and the total acids in the aqueous solution were determined (10·44 mequiv.). On evaporation of the remaining solution to dryness, calcium salts (1·537 g.) were obtained. The equivalent weight (147) derived from these results corresponds to a mixture of calcium glycollate tetrahydrate (81%) and Cannizzaro rearrangement products $(C_{14}H_{17}O_7)_2Ca$ (19%). Extraction of the mixture with hot absolute alcohol (200 ml.) yielded alcohol-soluble salts (0·42 g.), the benzaldehyde content (21·4%) of which corresponds to 64·1% of Cannizzaro rearrangement products in the alcohol-soluble fraction, or 17·5% in the total calcium salts. The products of acidic hydrolysis of the alcohol-soluble calcium salts were examined as follows:

(i) *Benzaldehyde.* A solution of the alcohol-soluble calcium salts (0·617 g.) in 0·1N-hydrochloric (100 ml.) was heated at 60—65° and the liberated benzaldehyde removed by a stream of nitrogen and collected in excess of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid (2—3 hr. required). Benzaldehyde 2 : 4-dinitrophenylhydrazone separated at 0° (0·368 g., 1·29 mmoles; m. p. and mixed m. p. 239—241°).

(ii) *Glycollaldehyde and glyoxylic acid.* The hydrolysis mixture after removal of the benzaldehyde was made 2N with respect to hydrochloric acid and a solution (30 ml.) of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid added. The mixture was heated for 2—3 hr. at 100° and kept at 0° overnight, and the precipitate (0·294 g.) was collected, washed with boiling alcohol, and identified as glyoxal bis-2 : 4-dinitrophenylhydrazone (0·103 g., 0·25 mmole), m. p. and mixed m. p. 323—325° (decomp.). Glyoxylic acid 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 193—195°, was isolated from the alcohol extract.

(iii) *Erythritol.* A further sample of the alcohol-soluble calcium salts (0·620 g.) was heated with 0·5N-sulphuric acid (100 ml.) at 100°, the benzaldehyde being removed by a stream of nitrogen. After 2 hr. the mixture was de-ionised by stirring with mixed Amberlite resins IRA-400(OH) and IR-120(H), and the solution on evaporation yielded erythritol (0·115 g., 0·96 mmole), m. p. and mixed m. p. 117—119°.

3-O-(D-1-*Methoxy*-2-*oxoethyl*)-D-*erythrose*.—2 : 4-*O*-Benzylidene-3-*O*-(D-1-methoxy-2-oxoethyl)-D-erythrose (31·6 g.) was suspended in 0·01N-sulphuric acid and heated, with stirring, at 70° for 1 hr. The mixture was cooled, unchanged material (0·3 g.) removed, and the aqueous layer extracted with ether to remove benzaldehyde. After neutralisation with Amberlite resin IR-4B(OH), the solution was evaporated to dryness, leaving a clear syrup (18·5 g.) which was shown by paper chromatography (solvent *a*, sprays *b* and *e*) to consist mainly of one component (R_F 0·70), with traces of a second component (R_F 0·39). When this mixture was

chromatographed on a carbon–Celite [15] column, erythrose was eluted by water (4 l.). Elution was then continued with 10% aqueous ethanol, and the desired erythrose derivative (R_F 0·70) was obtained as a clear colourless syrup (13·0 g.), $[\alpha]_D^{23}$ +88·0° (*c* 3·183 in H_2O) (Found: C, 43·7; H, 5·19; OMe, 15·3. $C_7H_{12}O_6$ requires C, 43·7; H, 6·2; OMe, 16·1%).

Reaction of 3-O-(D-1-*Methoxy*-2-*oxoethyl*)-D-*erythrose with Phenylhydrazine.*—(a) *Acid conditions.* The dialdehyde (0·40 g.) in 15% acetic acid (20 ml.) was heated at 100° for 3 min. with phenylhydrazine (2·0 g.). The yellow precipitate was glyoxal phenylosazone, m. p. and mixed m. p. 167—168° (after one crystallisation from alcohol–water). The filtrate was heated for a further 40 min. at 100° and the solid obtained (0·2 g.) was identified as erythrose phenylosazone, m. p. 162—164° (Found: C, 63·7; H, 6·7. Calc. for $C_{16}H_{18}O_2N_4$: C, 64·4; H, 6·0%).

(b) *Neutral conditions.* (i) The dialdehyde (0·2 g.) and sodium acetate (0·9 g.) were dissolved in water (10 ml.), and phenylhydrazine hydrochloride (0·5 g.) was added. The mixture was kept at room temperature for 5 min.; the oil which separated readily recrystallised from alcohol and was glyoxal phenylosazone, m. p. and mixed m. p. 167—169°.

(ii) Shaking the dialdehyde (0·3 g.) with water (20 ml.) and phenylhydrazine hydrochloride (0·7 g.) at room temperature for 5 min. gave glyoxal osazone, m. p. and mixed m. p. 166—168°.

Acid Hydrolysis of 3-O-(D-1-*Methoxy*-2-*oxoethyl*)-D-*erythrose.* The dialdehyde (0·21 g.) in 0·1N-sulphuric acid (50 ml.) was heated in a boiling-water bath for 5 hr. The rotation fell to a constant value and paper chromatography indicated erythrose, the R_F changing from 0·70 to 0·39 (solvent *a*; sprays, *b* and *e*). $[\alpha]_D^{20}$ +88° (initial); +35·2° (1 hr.); +2·5° (3 hr.); −1° (5 hr., const.).

Periodate Oxidation of 3-O-(D-1-*Methoxy*-2-*oxoethyl*)-D-*erythrose.*—The consumption of periodate by the dialdehyde (0·138 g.) in 0·004M-sodium metaperiodate (25 ml.), measured by reaction with acidic potassium iodide and titration with sodium thiosulphate, was 0·28 (10 min.), 0·42 (30 min.), 0·63 (2 hr.), 0·77 (5 hr.), 1·1 (24 hr.) mol.

Alkaline Degradation of 3-O-(D-1-*Methoxy*-2-*oxoethyl*)-D-*erythrose.*—(a) *Qualitative.* A solution of the dialdehyde (5·3 g.) in oxygen-free water (500 ml.) containing calcium hydroxide (2·5 g.) was kept at room temperature for 24 hr., then saturated with carbon dioxide and concentrated to *ca.* 100 ml. The distillate contained methanol but gave no reaction with 2 : 4-dinitrophenylhydrazine. The residual solution was filtered and the filtrate concentrated to a syrup (10—15 ml.), from which the calcium salts were precipitated by pouring the whole into alcohol–acetone (1 : 1; 200 ml.). The calcium salts were separated by filtration and extracted with dry methanol for 12 hr. in a Soxhlet extractor. The methanol extract was added to the alcohol–acetone solution, and the mixture was evaporated to dryness, yielding a syrup (0·7 g.) which was shown by paper chromatography (solvent *a*) to consist mainly of approximately equal amounts of unchanged material (R_F 0·70) and of an unidentified product (R_F 0·52; solvent *a*, sprays *b* and *e*; R_L 0·83, solvent *b*; L = lactic acid). Heating the neutral material with excess of sodium hydroxide removed the material of R_L 0·83 (solvent *b*), and some αγ-dihydroxybutyric and glycollic acid were formed.

Paper-chromatographic examination (solvent *b*) of the free acids derived from treatment with Amberlite resin IR-120(H) of the calcium salts after methanol-extraction showed five components, *viz.*, glycollic acid (R_L 0·80; sprays *a* and *b*), αγ-dihydroxybutyric acid (R_L 0·64; sprays *a* and *b*), and its lactone (R_L 1·00; sprays *b* and *f*), together with some neutral materials (R_L 0·83 in solvent *b*; R_F 0·52 and 0·70 in solvent *a*) and traces of a product (R_L 0·51) which reacted with spray *c*. After separation on thick paper, the glycollic acid was characterised as its 4-bromophenacyl ester, m. p. and mixed m. p. 138—140°, and αγ-dihydroxybutyric acid as its brucine salt, 169—171° (Found: N, 5·45. Calc. for $C_{27}H_{34}O_8N_2$: N, 5·45%). The same brucine salt was also obtained by fractional crystallisation of the mixed brucine salts from alcohol–ether.

Because of the difficulty of removing all the neutral material further examination of the acids was carried out after purification by means of ion-exchange resins as described for the quantitative experiments below. After purification in this way, paper chromatography showed the presence of all the compounds noted above except the neutral material (R_L 0·83). After the acid solution had been heated with Amberlite resin IR-120(H) for 1 hr. at 60°, the material of R_L 0·51 (solvent *b*) disappeared and a trace of a component corresponding to erythronolactone (solvent *a*; spray *f*) was detected.

(b) *Quantitative.* Oxygen-free nitrogen was passed through an aqueous solution (10 ml.) of the dialdehyde (0·1489 g.) and oxygen-free 0·0446N-calcium hydroxide (90 ml.) was added.

Samples (5·0 ml.) were withdrawn periodically and the alkali consumption determined in the usual way.[25]

Acids produced (equiv.)	0·14	0·65	0·96	1·23	1·37	1·47	1·56	1·63
Time (hr.)	0·05	0·33	1·0	3·5	24	72	168	384

In a separate experiment the dialdehyde (1·5247 g.) was dissolved in oxygen-free 0·04N-lime-water (1500 ml.). After 24 hr. the acids formed (12·45) were determined by treatment of an aliquot portion with Amberlite IR-120(H) resin (2·0 ml.), and the reaction was stopped by addition of excess of carbon dioxide. The mixture was concentrated to 500 ml., filtered from calcium carbonate, and the filtrate further concentrated to *ca.* 250 ml. A portion of the distillate (500 ml.) gave a very slight precipitate (5—10 mg.) with 2 : 4-dinitrophenylhydrazine reagent and gave no reaction with acidified Schiff's reagent.[26]

The aqueous reaction mixture was treated with Amberlite resin IR-120(H) (30 ml.) for 20—30 min. The resin was then removed by filtration and washed free from acid, and the combined filtrate and washings were stirred overnight with Amberlite resin IRA-400 (40 ml., carbonate form).[27] The resin, after transfer to a column containing a further 5 ml. of the same resin, was washed free from neutral products with distilled water (4 l.). Evaporation of the washings yielded a thick syrup (0·227 g.), which paper chromatography showed to consist of a number of products. The adsorbed acids were eluted from the resin with N-ammonium carbonate (2 l.). The solution of ammonium salts and excess of ammonium carbonate was concentrated to *ca.* 100 ml., heated at 65—70° for 1½—2 hr. to destroy any residual carbonate, and then diluted to 250 ml. This stock solution was then examined as follows.

The solution of ammonium salts (100 ml.) was stirred with Amberlite resin IR-120(H) (10 ml.) for 1 hr. The resin was separated by filtration and washed and the filtrate and washings were diluted to 250 ml. to give a standard solution of the free acids. Aliquot portions of this solution were used to determine the yields of total acids (12·25 mequiv.), volatile acids (1·22 mequiv.), and formic acid (0·98 mequiv.).

A further sample (100—150 ml.) of the solution of ammonium salts was concentrated to *ca.* 10 ml. and then shaken with Amberlite resin IR-120(H) (6 ml.) for 1 hr. The resin was separated by filtration and washed with a small amount of water. The strength of the acid solution (*ca.* 12 ml.) was determined and a suitable quantity (1·5—2 mequiv.) applied to Whatman No. 3 MM paper (22 in. × 24 in.), which was irrigated for 6—7 hr. with solvent *b*. The paper was dried for 40 hr. in a current of air at room temperature, and guide strips were cut off and developed in the usual way with spray *a* and, on the reverse side of the strip, spray *f*. The appropriate sections of the paper were then eluted with water, and the acids and lactones present determined by back-titration, the yield of glycollic acid being confirmed by Calkins's method.[22] In two determinations the ratio of $\alpha\gamma$-dihydroxybutyric acid (including lactone) to glycollic acid was 1·02 and 1·11. The total recovery, after allowance for guide strips and volatile acids, was 81%.

This work forms part of a programme of fundamental research undertaken by the Council of the British Rayon Research Association.

BRITISH RAYON RESEARCH ASSOCIATION,
HEALD GREEN LABORATORIES,
WYTHENSHAWE, MANCHESTER, 22.

[*Received, October 21st*, 1957.]

[25] Kenner and Richards, *J.*, 1954, 1784.
[26] Hoffpauir and Reeves, *Analyt. Chem.*, 1949, **21**, 815.
[27] Cf. Machell, *J.*, 1957, 3389.