

388. Characterisation of 3 : 4-Dimethyl D-Glucose.

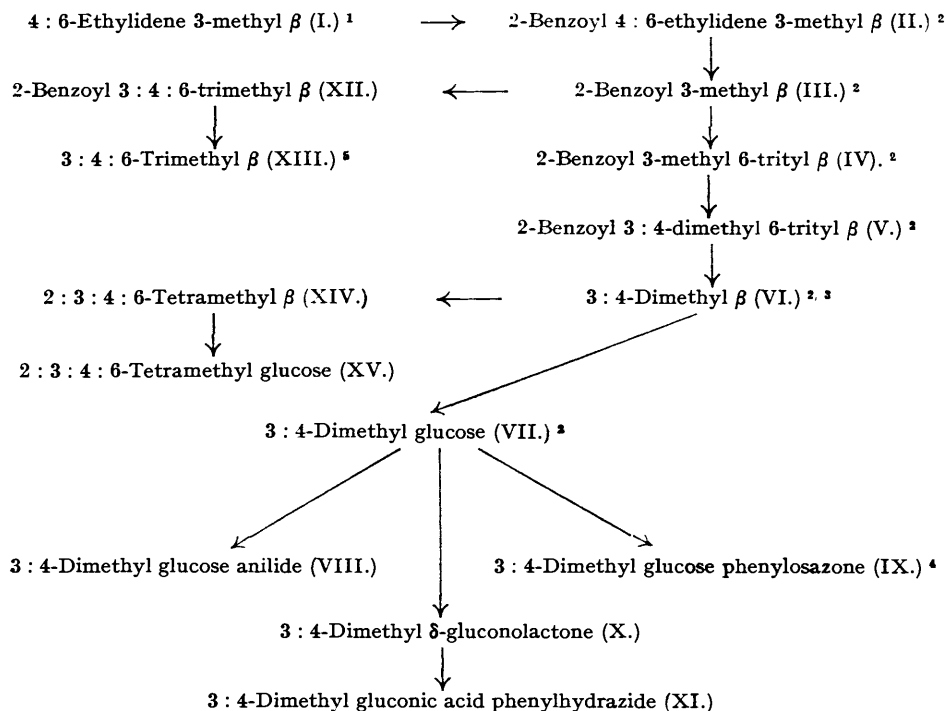
By D. J. BELL and G. D. GREVILLE.

3 : 4-Dimethyl D-glucose, first described by Dewar and Fort (*J.*, 1944, 496), has been synthesised by a somewhat different route and its constitution proved by (a) formation of a crystalline phenylosazone containing two methoxyl groups, (b) oxidation to the corresponding δ -lactone, characterised as the crystalline 3 : 4-dimethyl gluconic acid phenylhydrazide, and (c) periodate oxidation. During this oxidation the 1 : 2-link is attacked rapidly, yielding formic acid, and thereafter a slow production of formaldehyde ensues, presumably by fission of the 5 : 6-link. The sugar itself is most readily characterised as the crystalline anilide. The polarimetric behaviour of the sugar has been described incorrectly by Dewar and Fort.

SEVERAL years ago we started work on the synthesis of 3 : 4-dimethyl D-glucose, at that time unknown. We intended to attempt this through a partial acetolytic opening of the dioxan ring of 4 : 6-ethylidene 3-methyl β -methylglucoside (cf. Bell and Syngé, *J.*, 1937, 1711), but, although crystalline compounds were obtained at various later stages, these did not appear to have the desired structures, and the attempt was abandoned. Subsequently Dewar and Fort (*J.*, 1944, 496) described the synthesis of crystalline 3 : 4-dimethyl glucose starting with 4 : 6-ethylidene β -methylglucoside 2 : 3-dinitrate. These authors, likewise, encountered difficulty when attempting partial acetolysis of the ethylidene radical in their substances. Later work in this laboratory, however, rendered imperative the preparation of a quantity of

3 : 4-dimethyl glucose; we therefore recommenced our own work and, since Dewar and Fort gave no proof of the constitution of their sugar beyond that provided by their method of synthesis, we decided to characterise the sugar more fully.

The synthetic route adopted followed closely in its later stages that of Dewar and Fort. Certain improvements were introduced, notably (1) the use of the crystalline chloroform addition compound of 3-methyl β -methylglucoside as starting material for the preparation of its 4 : 6-ethylidene derivative (I), (2) the use of toluene during the working-up of the trityl compound (IV), and (3) the adoption of a totally different procedure for passing from (V) to (VI) (see following scheme, in which " β " denotes β -methylglucoside).



¹ Reeves, *J. Amer. Chem. Soc.*, 1944, **66**, 845. ² Dewar and Fort, *loc. cit.* ³ Dewar, Fort and McArthur, *J.*, 1944, 499. ⁴ McDonald and Jackson, *Bur. Stand. J. Res.*, 1940, **24**, 181. ⁵ Haworth, Hirst, and Panizzon, *J.*, 1934, 154; Sundberg, McClosky, Rees, and Coleman, *J. Amer. Chem. Soc.*, 1945, **67**, 1080.

It may be noted that the benzoyl radical occupying position 2 in (III) and (IV) does not migrate when these compounds are treated with Purdie's reagents; from (III), crystalline 3 : 4 : 6-trimethyl β -methylglucoside was obtained in good yield (this will be the subject of a future note with Dr. D. H. Northcote).

When we examined our specimen of crystalline 3 : 4-dimethyl glucose we found that, although it had the melting point recorded by Dewar and Fort, it showed very different polarimetric behaviour. Through the kind co-operation of Dr. J. Dewar we were able to re-examine the rotation of his original specimen; we find that it is indeed identical with our material. It is therefore clear that Dewar and Fort misquoted the polarimetric values, and also the direction of the mutarotation, in their report (see below).

Specific rotations of samples of 3 : 4-dimethyl D-glucose.

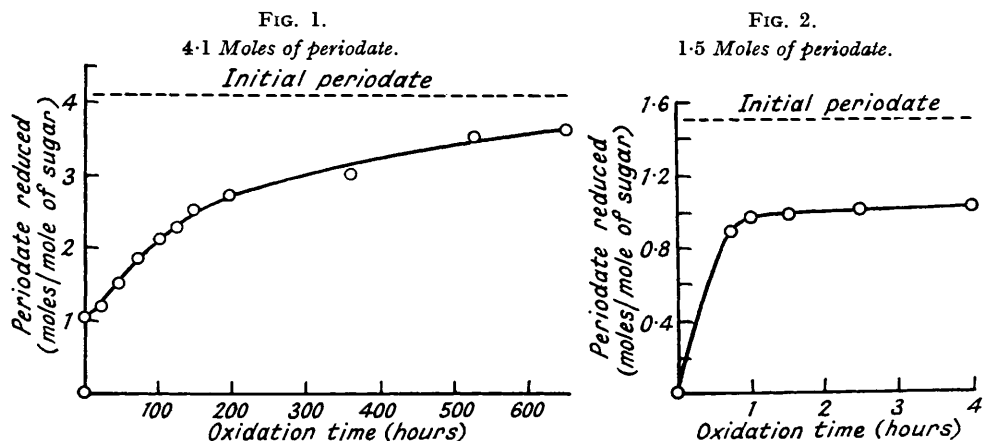
Source of sample.	$[\alpha]_D$ in water.	$[\alpha]_D$ in methanol.
Present authors	+93.5° (2.5 mins.) \longrightarrow	+96.1° (1 min.) \longrightarrow
	+77.8° (9.5 hrs., equilibrium)	+92.1° (6.8 hrs., equilibrium)
Dewar and Fort (by present authors)	+76.6° (3 mins.) \longrightarrow	+97.6° (4 mins.) \longrightarrow
	+76.6° (15 hrs.)	+92.0° (10 mins., equilibrium)
Dewar and Fort (published values)	+64.9° (10 mins.) \longrightarrow	+88.5° (6 mins.) \longrightarrow
	+94.8° (24 hrs.)	+102.2° (24 hrs.)

From the downward trend of the mutarotation it may be deduced that the crystalline sugar contains a preponderance of the α -anomer.

A convenient derivative for the identification of 3 : 4-dimethyl glucose is the crystalline anilide, which we have obtained in good yield. The phenylosazone can also be prepared; it was obtained in easily crystallisable form by seeding with material prepared from synthetic 3 : 4-dimethyl D-fructose and kindly provided by Dr. Emma J. McDonald of the U.S. Bureau of Standards.

Oxidation of the sugar with bromine water, followed by appropriate treatment, yielded a syrupy δ -lactone which, however, yields a readily crystallisable phenylhydrazide.

Oxidation of 3 : 4-dimethyl glucose by periodate in phosphate buffer (pH 7.4).



Experiments on the oxidation with periodate of 3 : 4-dimethyl glucose gave the following results: In bicarbonate buffer (pH 7.5) formation of formaldehyde was very slow, 0.55 mole being produced from one mole of sugar in 860 hours. In phosphate buffer (pH 7.4) (cf. Bell, *J.*, 1948, 992; Bell, Palmer, and Johns, *J.*, 1949, 1536) one mole of periodate was reduced rapidly, *i.e.*, in about one hour (Figs. 1 and 2). At this stage a negligible amount of formaldehyde was found, but one mole of formic acid had been liberated. There followed a slow, but fairly steady, reduction of periodate which was still proceeding when 3.5 moles had been reduced. 3 : 4-Dimethyl glucose, reacting in the open-chain form, would be expected to reduce only two moles of periodate, but it has been found that some other methylated sugars reduce more than the expected amount of periodate in phosphate buffer (Bell, Palmer, and Johns, *loc. cit.*). At 216 hours when 2.96 moles of periodate had been reduced, one mole of sugar had produced 0.84 mole of formaldehyde. With this sugar, therefore, periodate first attacks the 1 : 2-link yielding formic acid, and subsequently causes a slow production of formaldehyde, presumably by fission of the 5 : 6-link.

The constitution of the sugar was confirmed by the following: (1) Complete methylation of the glucoside (VI) yielded crystalline 2 : 3 : 4 : 6-tetramethyl β -methylglucoside, which on hydrolysis gave 2 : 3 : 4 : 6-tetramethyl glucose. (2) The formation of formic acid and formaldehyde by periodate oxidation is consistent with etherification in positions 3 and 4. (3) The sugar yields a dimethyl glucose phenylosazone, which is identical with that from 3 : 4-dimethyl fructose. (4) The lactone obtained after bromine oxidation behaves in solution as a member of the δ -series, confirming substitution of the hydroxyl group of position 4.

EXPERIMENTAL.

M. p.s are uncorrected unless otherwise stated. Rotations were measured in 2-dm. tubes. Solvents were removed under reduced pressure, and solvents distilled in glass were used for crystallisations. Elementary analyses were by Drs. Weiler and Straus, Oxford.

4 : 6-Ethylidene 3-Methyl β -Methylglucoside (I).—The crystalline chloroform addition compound of 3-methyl β -methylglucoside (Oldham, *J. Amer. Chem. Soc.*, 1934, 56, 1360) was dried to constant weight over calcium chloride in a desiccator continuously evacuated at *ca.* 15 mm. (4.5 hours). The product (21 g., equivalent to *ca.* 13 g. of the glucoside) was then shaken vigorously with 150 ml. of freshly-distilled paraldehyde containing 1% (v/v) of concentrated sulphuric acid and previously cooled to *ca.* 10°. After the mixture had been kept at 4°, and shaken at intervals, for 30 minutes most of the solid had dissolved.

The fluid was decanted into 200 ml. of 7.5% potassium carbonate solution, and the undissolved residue treated with a further 30 ml. of the paraldehyde-sulphuric acid mixture, dissolving completely in 15 minutes. The resulting fluid was then added to the potassium carbonate solution. The whole was then distilled under reduced pressure to remove unchanged paraldehyde, and the aqueous solution remaining was extracted 3 times with chloroform. The combined extracts were dried (Na_2SO_4) and evaporated, leaving a crystalline residue (16 g.) which after recrystallisation from ethanol-light petroleum (b. p. 40—60°) yielded (I) (11 g.), m. p. 133—134°, $[\alpha]_D^{25} - 43.5^\circ$ in chloroform (c, 4.6). Dewar and Fort (*loc. cit.*) give m. p. 134°, $[\alpha]_D^{25} - 43.7^\circ$ in chloroform; Reeves (*loc. cit.*) gives m. p. 133—134° (corr.). The residues from the recrystallisation yielded a crystalline substance, m. p. 96°, soluble in light petroleum (b. p. 40—60°), which has not yet been identified.

2-Benzoyl 4 : 6-Ethylidene 3-Methyl β -Methylglucoside (II).—The substance (I) (dried over phosphoric oxide) was dissolved with gentle warming in dry pyridine (4 moles); benzoyl chloride (2.3 moles) was added, the mixture being cooled in ice. Not more than 10 g. of the glucoside were treated at a time. After being kept at room temperature for 24 hours the mixture was stirred with water; 3 hours later the solid which had separated was filtered off and washed, first with much water, then with very dilute ammonia solution, and finally once more with water. Recrystallisation from 4 : 1 methanol-water gave (II), m. p. 157—159°, in 90% yield. Dewar and Fort (*loc. cit.*) give m. p. 159°.

2-Benzoyl 3 : 4-Dimethyl 6-Trityl β -Methylglucoside (V).—The above compound (II) was hydrolysed, following the instructions of Dewar and Fort (*loc. cit.*), to give 2-benzoyl 3-methyl β -methylglucoside (III), m. p. 139—140° (from carbon tetrachloride-chloroform). Dewar and Fort give m. p. 142—143°. The compound (III) (8.45 g.; dried in high vacuum) was heated, for 3 hours at 100° (air-bath temperature), with triphenylmethyl chloride (7.35 g., 0.975 mole) and dry pyridine (42 ml.) under an air-condenser fitted with a calcium chloride guard-tube. After cooling, the mixture was diluted with 250 ml. of toluene (chosen because the partition coefficient toluene/water for pyridine is low), and the whole poured into 4 l. of a 0.25% sodium hydrogen carbonate solution. The aqueous layer was separated and shaken with a little more toluene. The united toluene layers were again washed, first with sodium hydrogen carbonate solution and then with water, until the smell of pyridine was scarcely perceptible. After treatment with sodium sulphate and charcoal the toluene solution was filtered and evaporated. The residue (IV), a pale yellow glass, was dried in a high vacuum (yield, 14.35 g., 96%) and had $[\alpha]_D^{25} - 9.1^\circ$ in chloroform (c, 4.1). Dewar and Fort (*loc. cit.*) give $[\alpha]_D^{25} - 8.9^\circ$ in chloroform, yield 79%.

Methylation of (IV) with Purdie's reagents (Dewar and Fort, *loc. cit.*) yielded the 3 : 4-dimethyl derivative (V), m. p. 138—139°. Repeated recrystallisation from methanol failed to raise the m. p. above 139—140°. Dewar and Fort (*loc. cit.*) give m. p. 142°.

3 : 4-Dimethyl β -Methylglucoside (VI).—The compound (V) (8.0 g.) was dissolved in ethanol (36 ml.) under reflux and 35 ml. of 0.85N-aqueous sodium hydroxide (ca. 100% excess) added slowly. After 30 minutes' boiling the solution was cooled, 300 ml. of water were added, and the whole was extracted 3 times with chloroform. The combined extracts were washed with a little water, dried (Na_2SO_4), and evaporated. The resulting de-benzoylated product, a glass, was then heated under reflux with 90 ml. of acetone and 10 ml. of 0.1N-hydrochloric acid, the reaction being followed polarimetrically (7 hours). After cooling of the mixture more water was added, followed by an excess of silver carbonate. The solid material was removed by filtration (charcoal) and washed with water. The combined filtrate and washings were concentrated to remove acetone; trityl compounds which separated during this procedure were filtered off and washed with water. The filtrate and washings were combined and, after concentration, were extracted 3 times with benzene to complete the removal of trityl compounds. The aqueous solution was then evaporated, the residue dissolved in ethanol, and this solution filtered and evaporated below 50°. The product (VI) crystallised immediately when seeded; it weighed 2.70 g. after drying in a high vacuum. It was distilled at 0.05 mm. (bath-temperature ca. 150°) to give crystals, m. p. 80—83°, $[\alpha]_D^{25} - 12.3^\circ$ in chloroform (c, 5.1) [2.40 g., 77% from (V)]. Dewar and Fort (*loc. cit.*) give m. p. 79—81°, $[\alpha]_D^{25} - 11.9^\circ$ in chloroform, after recrystallisation.

2 : 3 : 4 : 6-Tetramethyl D-Glucose (XV) from (VI).—The glucoside (VI) (0.299 g.) was twice methylated with Purdie's reagents. The product crystallised, and after drying over sulphuric acid and solid sodium hydroxide at atmospheric pressure (the substance is volatile under reduced pressure at room temperature) weighed 0.33 g. (yield approx. theoretical) and had $[\alpha]_D^{25} - 17.3^\circ$ in water (c, 4.7). Irvine and Cameron (*J.*, 1905, 87, 900) give $[\alpha]_D^{20} - 17.3^\circ$ in water.

The crystals (0.2 g.) were hydrolysed by heating with N-hydrochloric acid at 95—100° for 5.5 hours. After appropriate treatment the hydrolysate yielded 0.15 g. of crystalline material. This was recrystallised from light petroleum (b. p. 40—60°), yielding 0.069 g. of 2 : 3 : 4 : 6-tetramethyl glucose, m. p. 86—92°, not depressed on admixture with authentic material of m. p. 90—92°, and having $[\alpha]_D^{18} + 82.4^\circ$ in water (c, 2.05) at equilibrium. Haworth, Long, and Plant (*J.*, 1927, 2809) give $[\alpha]_D^{18} + 83.0^\circ$. The mother-liquors from the recrystallisation were evaporated, leaving a crystalline residue. On a paper chromatogram (n-butanol-water) this residue gave only one spot. R_F values were: recrystallised material, 79.5; residue 79; authentic material 79.5.

3 : 4-Dimethyl Glucose (VII).—The glucoside (VI) was hydrolysed in 5% solution in N-hydrochloric acid at 95—100°, the reaction being followed polarimetrically (5.5 hours). The solution was treated with a small excess of silver carbonate and filtered (charcoal—"Celite") before and after passage of hydrogen sulphide. The solution was then evaporated below 50°, and the residue dissolved in ethanol, filtered, and evaporated. Two recrystallisations of the residue from dry ethyl acetate yielded (VII), m. p. 110—113°. Dewar and Fort (*loc. cit.*) give 113°. The m. p. was not depressed on admixture with a specimen (m. p. 110—112°) from Dr. Dewar. The sugar was dried to constant weight in a high vacuum at room temperature (Found: C, 46.2; H, 7.55. Calc. for $\text{C}_6\text{H}_{14}\text{O}_6$: C, 46.2; H, 7.7%). For rotation, see Table (p. 1903). Dr. Dewar's specimen contained a small amount of an impurity insoluble in propyl acetate, and after recrystallisation from this solvent had $[\alpha]_D^{18}$ in methanol (c, 0.45) + 97.6° (4 minutes), + 92.0° (10 minutes, equilibrium value).

Our material gave a single compact spot on a paper chromatogram (n-butanol-water or ethyl acetate-water).

3 : 4-Dimethyl Glucose Anilide (VIII).—The sugar (VII), in 5% solution in dry ethanol, was refluxed with aniline (1.5 moles) for 5 hours. Evaporation of the solvent and recrystallisation of the residue, first from *n*-butanol–light petroleum (b. p. 40–60°), then from ethanol, gave the *anilide* (VIII) as colourless needles, m. p. 177–178° (not raised by further recrystallisation from ethanol), $[\alpha]_D^{20}$ in ethanol (*c.* 2.2) –106° (17 minutes), –106° (17 hours) (Found: C, 59.2; H, 7.5; N, 4.85; OMe, 21.4. $C_{14}H_{21}O_8N$ requires C, 59.4; H, 7.4; N, 4.95; OMe, 21.9%).

3 : 4-Dimethyl Glucose Phenylsazone (IX).—A solution of the sugar (VII) in dilute acetic acid was heated for 75 minutes at 100° with phenylhydrazine (*ca.* 4.5 moles) and a little sodium hydrogen sulphite (Hamilton, *J. Amer. Chem. Soc.*, 1934, 56, 487). The oil which separated crystallised when seeded with the phenylsazone from 3 : 4-dimethyl fructose, kindly supplied by Dr. Emma J. McDonald. The crystals were washed thoroughly with water and dried in a high vacuum over phosphoric oxide. After two recrystallisations (charcoal) from ether–light petroleum (b. p. 60–80°) the yellow needles (IX) had m. p. 126–127° (Found: C, 62.2; H, 6.9; N, 14.6; OMe, 16.4. Calc. for $C_{20}H_{26}O_8N_4$: C, 62.2; H, 6.75; N, 14.5; OMe, 16.1%). Another preparation had m. p. 120°, unchanged by further recrystallisation. It is noteworthy that the phenylsazone from 3 : 4-dimethyl fructose (obtained from irisin; forthcoming communication), which showed no depression of m. p. on being mixed with (IX), melted at 126°, but had m. p. 120° after further recrystallisation. McDonald and Jackson (*loc. cit.*) obtained two specimens of the phenylsazone from synthetic 3 : 4-dimethyl fructose with m. p. 126° and a third with m. p. 121° (no analyses given). Haworth, Hirst, and Isherwood (*J.*, 1937, 784) prepared the phenylsazone from 3 : 4-dimethyl mannose but were unable to determine a m. p.

3 : 4-Dimethyl δ -Gluconolactone (X).—The sugar (VII), in 5% solution in water, was oxidised by bromine at room temperature, a small amount of undissolved bromine being kept present throughout. After 30 hours Fehling's test was negative. The solution was then neutralised with a slight excess of silver carbonate (free from alkali and alkaline-earth metals) and filtered (charcoal—"Celite") before and after hydrogen sulphide had been passed in. It was evaporated, leaving a syrupy residue which was distilled at 0.01 mm. (bath-temperature *ca.* 150°; receiving surface cooled with acetone–carbon dioxide), yielding 3 : 4-dimethyl gluconolactone (X) as a colourless syrup (Found: OMe, 29.85. $C_8H_{14}O_6$ requires OMe, 30.1%), n_D^{20} 1.4862 (decrement for rise of 1°, 0.00028 from 19° to 29°), $[\alpha]_D^{25}$ in water (*c.* 1.2) +70.8° (9 minutes), +57.2° (70 minutes), +30.5° (220 minutes), +20.2° (350 minutes), +16.4° (10.5 hours), +16.0° (22 hours, equilibrium value). The rate of hydrolysis indicates a δ -lactone.

3 : 4-Dimethyl Gluconic Acid Phenylhydrazide (XI).—The lactone (X) (0.33 g.) was dissolved under reflux in dry benzene (9 ml.) with the aid of a few drops of absolute ethanol. Phenylhydrazine (0.28 ml., *ca.* 2 moles) was added and the solution boiled for 5 hours. It was then evaporated; the residue crystallised on being warmed with dry ether. The crystals were washed with small quantities of dry ether and of ether–light petroleum (b. p. 80–100°). Recrystallisation from ethanol–ether–light petroleum (b. p. 80–100°) gave the *phenylhydrazide* (XI) as colourless needles, m. p. 119° (not raised by further recrystallisation) (0.33 g., 66%), $[\alpha]_D^{25}$ +27.1° in ethanol (*c.* 1.6) (Found: C, 53.55; H, 7.0; N, 9.45; OMe, 20.1. $C_{14}H_{22}O_8N_2$ requires C, 53.5; H, 7.0; N, 8.9; OMe, 19.7%).

Oxidation of 3 : 4-Dimethyl Glucose by Periodate.—The oxidations were done at room temperature. The amounts of periodate reduced, and of formaldehyde and formic acid formed, are expressed as moles (or g.-equivalents) per mole of sugar initially present. Simultaneous control determinations on the reagents were always carried out and the necessary slight corrections made.

(a) *In bicarbonate buffer.* The formaldehyde formed when the sugar, in 9mm-solution, was treated with periodate (6.7 moles) in bicarbonate buffer (pH 7.5) was determined according to Reeves (*J. Amer. Chem. Soc.*, 1941, 63, 1476). Formaldehyde was produced very slowly. The formaldehyde–dimedon compound had m. p. 193° (corr.).

Oxidation time (hrs.)	22	96	860
Formaldehyde produced (moles per mole)	0.09	0.24	0.55

(b) *In phosphate buffer.* Periodate was determined by titration of samples of the reaction mixture with 0.02N-sodium arsenite after addition of water and potassium iodide. In the experiment shown in Fig. 1 the sugar, in 4mm-solution, was oxidised with periodate (4.1 moles) in 0.025M-sodium phosphate at pH 7.4. 1.07 Moles of periodate were reduced in the first 2.5 hours; thereafter there was a slow but fairly steady reduction, still proceeding at 600 hours. The initial rapid reduction was examined more closely in another experiment (Fig. 2) in which the sugar, in 15mm-solution, was treated with 1.5 moles of periodate; 1 mole of periodate was reduced in about 1 hour.

Formaldehyde production was studied as follows: The sugar, in 6mm-solution, was oxidised with periodate (3.4 moles) in 0.03M-sodium phosphate at pH 7.4. Samples (10 ml.) were removed at intervals and treated with *N*-hydrochloric acid (3 ml.) and 1.2M-potassium arsenite (B.D.H.) (1 ml.). When the liberated iodine had disappeared, bromocresol-green was added, followed by *M*-sodium acetate until the solution was blue-green. The formaldehyde was then determined by weighing the dimedon compound, m. p. 188–189° (corr.), as described by Bell (*loc. cit.*). The results are given below. It is seen that the

Oxidation time (hrs.)	2	95	216
Periodate reduced (moles per mole)	1.05	2.28	2.96
Formaldehyde produced (mole per mole)	0.005	0.64	0.84

initial reduction of 1 mole of periodate is accompanied by negligible production of formaldehyde, but that during the subsequent reduction of more than the expected amount of periodate formaldehyde is slowly released.

Production of formic acid during the first few hours was determined as follows (cf. Bell, Palmer, and Johns, *loc. cit.*). The sugar, in 16mm-solution, was oxidised with periodate (1.48 moles) in 0.03M-phosphate at pH 7.5. Samples (2 ml.) of the reaction mixture were withdrawn at intervals, treated with purified ethylene glycol (0.5 ml.) and after 1 hour diluted to 10 ml. with 10% potassium hydrogen sulphate. Samples (3 ml.) of this solution were then pipetted into the steam-distillation

apparatus (Markham, *Biochem. J.*, 1942, **36**, 790); 65 ml. of distillate were collected and titrated with 0·01N-sodium hydroxide (carbon dioxide-free) in a stream of carbon dioxide-free air, with phenol-red as indicator. The blank determination on the reagents was 0·015 ml. of sodium hydroxide, corresponding to 0·02 g.-equiv. of volatile acid per mole of sugar. Distillations were run in duplicate. Control determinations on formic acid put through the whole process showed a recovery of approx. 99%. The results are given below.

Oxidation time (mins.)	45	75	240
Periodate reduced (moles per mole)	0·99	1·02	1·07
Volatile acid formed (g.-equivs. per mole)	0·94	0·99	1·01

Evidence that the volatile acid was in fact formic acid was obtained as follows: The distillates, made slightly alkaline with sodium hydroxide, were evaporated (Pirie, *Biochem. J.*, 1946, **40**, 100) and the residues heated in glass-stoppered tubes with 2 ml. of 5% mercuric sulphate in 0·5N-sulphuric acid for 1 hour at 100°. The resulting suspensions were distilled and titrated as before. Alternatively, the dried residues were dissolved in 3 ml. of the acid mercuric sulphate solution and distilled, slowly at first, without preliminary heating. Both methods resulted in quantitative elimination of the volatile acid.

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