360. *Reactions with Periodate of 2 : 3 : 4 : 6-Tetramethyl* D-*Glucose, the Trimethyl* D-*Glucopyranoses, and Other Methoxy-compounds.*

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Reactions of all four trimethyl D-glucopyranoses with metaperiodate at pH 5 have been studied; 3 : 4 : 6-trimethyl glucose can be determined in presence of the others since it alone yields volatile acid (one equivalent). Unexpectedly, 2 : 3 : 6-trimethyl and 3 : 4-dimethyl D-glucose resist attack by metaperiodate at $C_{(5)}$ and the adjacent unmethylated carbon atom.

In carbohydrate derivatives periodate reacts with (i) α-glycol groups, (ii) active hydrogen groups, (iii) some structures lacking (i) (actual or potential) and (ii). Type (iii) reactions with some methylated carbohydrate derivatives have been studied. They occur at pH 7·5 but only very slowly at pH 5; they are not dependent on hydroxyl groups, and their rate increases with the number of methoxyl groups. Not all methoxy-compounds react at pH 7·5.

2 : 3 : 4-Trimethyl D-glucose, purified chromatographically, had $[\alpha]_D^{20}$ +78·7° in water, 10° higher than any previously recorded value.

Reactions with periodate have become important in analyses of polysaccharides and methylated monosaccharides obtained from them. The reactions with many of the latter have been insufficiently studied; investigations on D-glucose derivatives are reported here. Rates of reaction of periodate and the nature of the products depend on pH. Many methylated monosaccharides fail to give the expected amount of formaldehyde with periodic acid, but the yield is increased with periodate buffered at pH 7·5 (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476; Jeanloz, *Helv. Chim. Acta*, 1944, **27**, 1509; Bell, *J.*, 1948, 992; Bell, Palmer, and Johns, *J.*, 1949, 1536). The periodate consumption then exceeds, often greatly, that required by the α-glycol groupings (" over-consumption ") (Bell *et al.*, *loc. cit.*; Bell and Greville, *J.*, 1950, 1902). Thus the increased formaldehyde production accompanies, and probably depends upon, attack on groups other than the primary alcohol group and its neighbour; and, in general, periodate at pH 7—8 attacks parts of the molecule lacking hydroxyl groups owing to methylation or glycoside formation (Grangaard, Gladding, and Purves, *Paper Trade J.*, 1942, **115**, No. 7, 41; also Jeanloz and Forchielli, *Helv. Chim. Acta*, 1950, **33**, 1690; *J. Biol. Chem.*, 1951, **188**, 361; **190**, 537). Demethylation or cleavage of the chain may well occur.

It is thus convenient to distinguish at least three types of reaction of periodate with carbohydrates and their derivatives: (i) Reactions with –CH(OH)·CH(OH)– and –CH(OH)·CO–, –CH(OH)·$CH(NH_2)$–, and related groups (" Malapradian reactions "). (ii) Oxidations at active hydrogen groups (Sprinson and Chargaff, *J. Biol. Chem.*, 1946, **164**, 433), *e.g.*, in structures left after Type (i) oxidations of hexofuranosides and uronides (Huebner, Lohmar, Dimler, Moore, and Link, *J. Biol. Chem.*, 1945, **159**, 503; Halsall, Hirst, and Jones, *J.*, 1947, 1427) and of maltose and amylose (Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488). (iii) Reactions with compounds, or parts of compounds,

lacking groupings involved in (i) and (ii), *e.g.*, methylated carbohydrate derivatives without actual or potential α-glycol structure [Type (ii) or (iii) reactions occurring with Type (i) have usually been designated " over-oxidation "].

We have studied at 22° (*a*) the reaction of metaperiodate (pH 5) with tri- and tetramethyl glucopyranoses, and (*b*) the occurrence and velocity, with various methylated derivatives, of Type (iii) reactions. It may be pointed out that precautions are usually taken to minimise Type (iii) reactions in analytical uses of periodate (Halsall *et al.*, *loc. cit.*; Potter and Hassid, *loc. cit.*). Certain terms used in this paper are thus defined :

" Metaperiodate " : aqueous $NaIO_4$ (pH 5).

" Neutral periodate " : aqueous periodate at pH 7·5—7·6.

" Moles of periodate " : moles of periodate per mole of sugar initially present.

" Volatile acid " : acid steam-distilled from solutions acidified with potassium hydrogen sulphate.

" Periodate consumed " : periodate disappearance measured solely by arsenite titration at pH 7·5.

Note. Hughes and Nevell (*Trans. Faraday Soc.*, 1948, **44**, 1941) found that metaperiodate reacting with glucose is partly converted, without reduction, into a form unreactive with arsenite in presence of sodium hydrogen carbonate.

Reactions with Metaperiodate.—With 2 : 3 : 6- and 2 : 4 : 6-trimethyl glucose only 0·05 mole of metaperiodate is consumed in 200 hours (Expt. 1). The low reactivity of the 2 : 3 : 6-compound has already been noted by Palmer (Thesis, Cambridge, 1951) with periodate in phosphate buffer pH 7·5. 2 : 3 : 4-Trimethyl glucose reacts more rapidly than the above two sugars, but still slowly (0·8 mole of metaperiodate in 200 hours) (Expt. 1). The progress of the reaction is similar to that noted in potassium phthalate buffer of pH 6 (Bell *et al.*, *loc. cit.*), but differs in that the periodate consumption goes beyond one mole. This difference may be due to the precipitation of potassium periodate by the buffer used by Bell *et al.* (cf. Halsall *et al.*, *loc. cit.*). 3 : 4 : 6-Trimethyl glucose rapidly consumes one mole of metaperiodate (unchanged up to 90 hours) (Expt. 2). One equivalent of a volatile acid, almost certainly formic, is found (Expt. 3). 2 : 3 : 4 : 6-Tetramethyl glucose unexpectedly consumes metaperiodate slightly faster than the 2 : 3 : 6- and the 2 : 4 : 6-analogue (Expt. 6).

The 3 : 4 : 6- and the 2 : 4 : 6-compound behave as expected. It is peculiar that 2 : 3 : 6-trimethyl glucose reacts no more rapidly than does the 2 : 4 : 6 isomer. In an aldohexose, carbon atom 5 will carry a hydroxyl group only when the sugar is in the aldehyde or the furanose form, and Jeanloz (*loc. cit.*) suggested that 2 : 3 : 4-trimethyl glucose reacts slowly with metaperiodate because it cannot assume the furanose configuration. A similar consideration may apply to 2 : 3 : 6-trimethyl glucose, which also can have an α-glycol structure only in the aldehyde form. Two compounds are known which, although they possess this structure, are unattacked; these are 1 : 6-anhydro-β-D-glucofuranose (Dimler, Davis, and Hilbert, *J. Amer. Chem. Soc.*, 1946, **68**, 1377) and 1 : 6-anhydro-α-D-galactofuranose (Alexander, Dimler, and Mehltretter, *ibid.*, 1951, **73**, 4658). This resistance was ascribed either to the *trans*-configuration of the hydroxyl groups or to the double lactol ring system. The former explanation is the less likely, since 1 : 6-anhydro-β-glucopyranose is attacked quite rapidly by metaperiodate (Jackson and Hudson, *J. Amer. Chem. Soc.*, 1940, **62**, 958; also see Expt. 8).

With 3 : 4-dimethyl glucose (Expts. 2 and 5), only one mole of metaperiodate is consumed, no formaldehyde appears, and one equivalent of volatile acid is found. The immunity of $C_{(5)}$ and $C_{(6)}$ may only partly be due to the impossibility of the sugar's assuming the furanose form, for when it has reacted, no titratable acid is present in the solution (also with 3 : 4 : 6-trimethyl glucose). Probably the reaction with metaperiodate yields a product still containing six carbon atoms :

3 : 4-Dimethyl glucose $\xrightarrow{NaIO_4}$ 6-carbon compound $\xrightarrow{H^+}$ $H{\cdot}CO_2H$ + (?) 2 : 3-dimethyl arabinose.

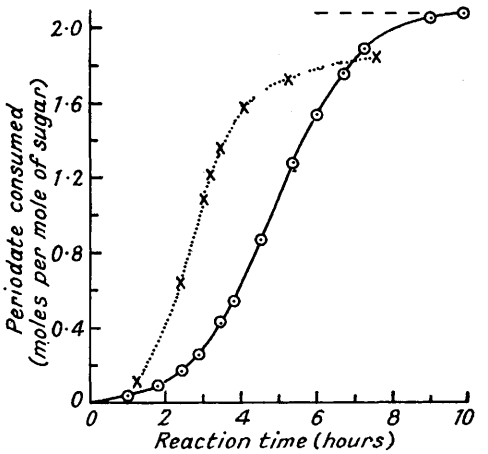
This will be the subject of a future communication. The product is rather stable in metaperiodate solution, and if it is the cyclic diester of periodic acid suggested by Criegee

(*Chem. Abs.*, 1935, **29**, 6820; see also Hughes and Nevell, *loc. cit.*) or, more likely, the formic ester (Halsall *et al.*, *loc. cit.*; Meyer and Rathgeb, *Helv. Chim. Acta*, 1949, **32**, 1102), there will be no adjacent hydroxyl group unless it breaks down. Even then the resulting dimethyl pentose is likely to be as resistant as 2 : 3 : 4-trimethyl glucose, since they have the same structure about their four highest-numbered carbon atoms.

The different behaviour of the four trimethyl glucopyranoses towards metaperiodate allows the 3 : 4 : 6-compound to be determined in the presence of the others by measurement of the volatile acid after 12 hours (Expt. 4). The method is still applicable after 60 hours' treatment. Previously, this sugar had been determined in presence of the others by weighing the hydrazodicarbonamide (Barker, Hirst, and Jones, *J.*, 1938, 1695; Granichstädten and Percival, *J.*, 1943, 54), and also by chromatographic separation of the *p*-phenylazobenzoyl derivatives of the glucitols (Boissonnas, *Helv. Chim. Acta*, 1947, **30**, 1689). The periodate method is simple and accurate.

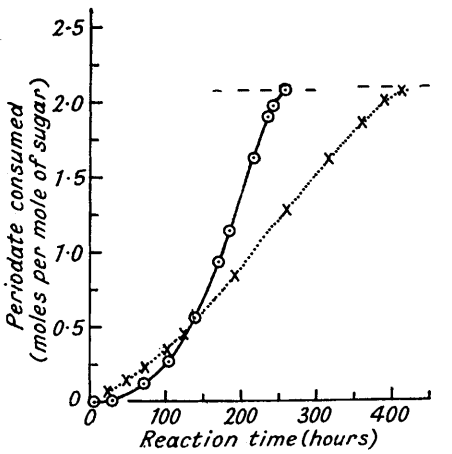
Reactions with Neutral Periodate.—In phosphate at pH 7·5, 2 : 3 : 6-trimethyl glucose is slowly attacked by periodate (1 mole in about 200 hours), consumption continuing to

FIG. 1. 2 : 3 : 4-*Trimethyl glucose with* 2·08 *moles of periodate.*



⊙ *In phosphate,* pH 7·5 (*Expt.* 12).
× *Without buffer,* pH 7·6 (*Expt.* 18).

FIG. 2. *Reactions in phosphate,* pH 7·5.



⊙ 2 : 4 : 6-*Trimethyl glucose with* 2·09 *moles of periodate* (*Expt.* 11).
× 2 : 3 : 6-*Trimethyl glucose with* 2·11 *moles of periodate* (*Expt.* 10).

(*Horizontal broken lines show initial periodate.*)

3 moles or more (Fig. 2, Expt. 10). Since no break in the curve appears near one mole, the attack probably occurs simultaneously at several points. The 2 : 4 : 6-compound consumes periodate somewhat more rapidly (Fig. 2, Expt. 11). It is remarkable that this compound should react more rapidly than one with potential α-glycol structure. With 2 : 3 : 4 : 6-tetramethyl glucose there is a further increase in speed (1 mole in about 100 hours) (Expt. 14). With these compounds which are scarcely attacked by metaperiodate, the rate in phosphate at pH 7·5 is not even dependent on the number of actual or potential hydroxyl groups, but rather on the number of methoxyl radicals.

14 Moles of periodate are needed to oxidise tetramethyl glucose completely to formic acid, and 24 to carbon dioxide. Periodate consumption ceases when 14·5 have been used (Expt. 14). Some volatile acid (almost certainly formic) appears at first, but nearly disappears by the end of the reaction. With the corresponding glucoside the reaction is still continuing slowly when 15 moles of periodate have been consumed; very little volatile acid is then found (Expt. 15).

2 : 3 : 4-Trimethyl glucose reacts with metaperiodate considerably faster than the 2 : 3 : 6- or the 2 : 4 : 6-compound, and in phosphate the speed (1 mole in about 5 hours) is

also of a totally different magnitude (Fig. 1; cf. Bell *et al.*, *loc. cit.*). Type (iii) reactions may thus be as rapid as Type (i). There is no break in the curve near one mole, hence no preferential Type (i) attack at $C_{(5)}$ and $C_{(6)}$. This explains the appearance of formic acid together with less than one mole of formaldehyde (Bell *et al.*, *loc. cit.*). With 3 : 4 : 6-trimethyl glucose, the rapid disappearance of one mole of periodate is followed by a slower but steady consumption (Expt. 13, i), due only in minor degree to oxidation of the formic acid (Expts. 13, ii and 17). With 3 : 4-dimethyl glucose the second stage is more rapid (Expt. 13, iii; cf. Bell and Greville, *loc. cit.*). Since the intermediate compound already mentioned decomposes in a few hours, the *initial* oxidation of 3 : 4 : 6-trimethyl glucose should lead to 2 : 3 : 5-trimethyl arabinose, and that of 3 : 4-dimethyl glucose to 2 : 3-dimethyl arabinose. These products resemble, in distribution of methyl radicals, 2 : 4 : 6- and 2 : 3 : 4-trimethyl glucose, respectively. As the latter reacts much faster than the former, the more rapid second stage with 3 : 4-dimethyl glucose is understandable.

The presence of a free reducing group is not necessary for Type (iii) reactions, as is shown by 3 : 4-dimethyl, 3 : 4 : 6-trimethyl, and 2 : 3 : 4 : 6-tetramethyl β-methylglucosides (Expt. 15, i); the last-named has no hydroxyl group at all. Again, the rate of consumption of periodate increases with the number of methyl groups.

To test whether periodate in phosphate at pH 7·5 will attack any monomethyl α-glycol or simple methyl ether, 2-methoxyethanol and *n*-butyl methyl ether were chosen. Neither

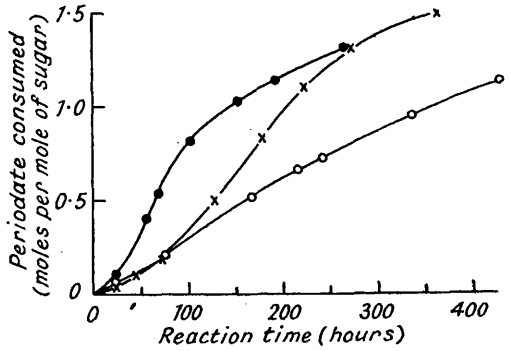


FIG. 3. *Reactions with periodate* (2·1 *moles*) *initially at* pH 7·6, *without buffer addition* (*Expts.* 19, 20).

○ 2 : 3 : 6-*Trimethyl glucose.*
× 2 : 4 : 6-*Trimethyl glucose.*
● 2 : 3 : 4 : 6-*Tetramethyl glucose.*

consumed periodate (Expt. 16). Even some methylated derivatives of monosaccharides do not react; *e.g.*, 2-benzoyl 3 : 4 : 6-trimethyl β-methylglucoside and 2 : 3 : 4-trimethyl 1 : 6-anhydro-β-glucose (Expt. 16). The 1 : 6-anhydro-ring is no bar to action of periodate on α-glycols, since 1 : 6-anhydro-β-glucopyranose reacts rapidly.

In no reaction, with or without phosphate, have we seen evidence of free iodine.

Most of these reactions in phosphate proceed with increasing velocity. For example (Figs. 1 and 2), the velocity increases until about two-thirds of the periodate have disappeared, and then decreases with the fall in periodate concentration. It seemed possible that the reaction might be affected by the surface of the vessel; but the time course of the reaction was unaltered either in a flask lined with paraffin wax or when fine glass particles ("Ballotini" No. 12) were added. Possibly, acceleration of periodate consumption is due to increasing numbers of points of attack from the disruption of the molecule; but the second stage with 3 : 4-dimethyl and 3 : 4 : 6-trimethyl glucose (see above) does not show acceleration (Expt. 13).

Bell *et al.* (*loc. cit.*) suggested that the chemical nature of the buffer can influence the course of reactions with neutral periodate. But when 2 : 3 : 6-trimethyl, 2 : 4 : 6-trimethyl, and 2 : 3 : 4 : 6-tetramethyl glucose are treated with periodate brought initially to pH 7·6 with sodium hydroxide, with no extra buffer, the rate with each sugar is about the same, during the first 100—150 hours, as in presence of phosphate, but then falls with increasing acidity (Fig. 3). With 2 : 3 : 4-trimethyl glucose the initial rate is even greater than in phosphate (Fig. 1). The pH changes with the 2 : 3 : 6-trimethyl sugar again suggest no preferential attack at $C_{(4)}$ and $C_{(5)}$ (Expt. 19).

Type (iii) reactions also proceeded, but very slowly, at pH 5; compounds which reacted with neutral periodate all underwent some reaction with metaperiodate, some even showing typical acceleration (Expt. 7). Of compounds not reacting at pH 7·5, those tested were also unattacked at pH 5. It may be that $H_3IO_6^{--}$ is more effective for Type (iii) reactions than $H_4IO_6^-$, since the former is absent from metaperiodate solutions.

Experimental

M. p.s (uncorr.) and rotations (measured in 2-dm. tubes) were determined on specimens dried over phosphoric oxide *in vacuo* at room temperature. Evaporations were done under reduced pressure, and solvents distilled in glass were used for recrystallisations. Paper chromatograms, 2% sugar solutions being used, were run with *n*-butanol–water on Whatman No. 1 papers at room temperature and sprayed with aniline hydrogen phthalate. pH was measured by the glass electrode.

2 : 3 : 4-*Trimethyl Glucose.*—2 : 3 : 4-Trimethyl 1 : 6-anhydro-β-glucose, purified to constant m. p. (see table below), was hydrolysed (5% solution in N-hydrochloric acid) at 95—100° to constant rotation (8 hours). After isolation (silver carbonate method) the sugar, dried at 0·05 mm. for 8 hours, had $[\alpha]_D^{20}$ +70·4° in water at equilibrium. From the constancy of the rotation, such drying did not cause anhydride formation. The latter occurs (Irvine and Oldham, *J.*, 1921, **119**, 1744) during the usual purification by distillation, so partition chromatography (Bell, *J.*, 1944, **473**; Bell *et al.*, *loc. cit.*) was used. Anhydro-compound (about 9%) was removed by elution with chloroform (7 column-lengths). The sugar was eluted with *n*-butanol–chloroform and, after removal of solvents, dissolved in ether, filtered, evaporated, and dried as before. The syrup (about 83%) had $[\alpha]_D^{20}$ +78·7° in water (*c*, 2·5) at equilibrium, n_D^{20} 1·4700 (decrement for rise of 1°, 0·00035 from 20° to 25°). This rotation is more than 10° higher than any previously recorded (for references, see Bourne and Peat, *Adv. Carbohydrate Chem.*, 1950, **5**, 145). Reduction of hypoiodite was determined under Macleod and Robison's conditions (*Biochem. J.*, 1929, **23**, 517) but on a larger scale (14 mg. of sugar); complete in 30 minutes, it was 100·0% of the theoretical. As in addition the product gave a single compact spot on paper chromatograms, its $[\alpha]_D$ is probably near that of the pure sugar.

Other Compounds.—All sugars gave single spots on paper chromatograms. A hydrolysate of the tetramethyl glucoside contained trimethyl sugar (considerably less than 1%, by paper chromatography); this possibly arose during hydrolysis. Characteristics were (G = glucose; M = β-methylglucoside; eqm. = equilibrium):

Compound	M. p.	$[\alpha]_D$ (water)
2 : 3 : 6-Trimethyl G	109—113°	+68·9° (18°, eqm.)
2 : 4 : 6-Trimethyl G	118—120	+77·0° (16°, eqm.)
2 : 3 : 4 : 6-Tetramethyl G	90—92	+81·5° (19°, eqm.) [a]
2 : 3 : 4 : 6-Tetramethyl M	36—37	−18·2° (19°) [b]
1 : 6-Anhydro-glucopyranose	181—182	−66·9° (18°)
2 : 3 : 4-Trimethyl 1 : 6-anhydro-β-G	59—60 [c]	−62·3° (18°)
2-Methoxyethanol [d]	124·0—124·3 (b. p.)	—
n-Butyl methyl ether [d]	68—69 (b. p.)	—

For 3 : 4-dimethylglucose and β-methylglucoside, see Bell and Greville (*loc. cit.*). For 3 : 4 : 6-trimethyl glucose, its β-methylglucoside, and 2-benzoyl 3 : 4 : 6-trimethyl β-methylglucoside, see Greville and Northcote (*J.*, 1952, 1957).

[a] Fits West and Holden's equation (*Org. Synth.*, 1940, **20**, 97). [b] n_D^{23} 1·4394 (decrement for rise of 1°, 0·00040 from 23° to 33°). [c] Note difference from literature values. [d] Peroxide-free.

Reactions with Periodate.—Procedures. Reactions were carried out in glass-stoppered volumetric flasks in the dark, and, unless otherwise stated, in a thermostat at 22°. Simultaneous control determinations on the reagents were always carried out. Glass-distilled water and "AnalaR" reagents were used. The crystalline $NaIO_4$ contained no free acid (for test, see Halsall *et al.*, *loc. cit.*). Unless otherwise stated, sugars were dissolved in water immediately before the reaction mixture was set up. Reactions were carried out (*a*) with metaperiodate (0·02M, pH 5·1); (*b*) with periodate at pH 7·5 in phosphate buffer (0·025M-sodium phosphate pH 7·5, 0·01M-$NaIO_4$, and 0·003N-sodium hydroxide); and (*c*) with periodate, initially pH 7·6, without addition of buffer, the solution containing 0·01M-$NaIO_4$ with the requisite sodium hydroxide (0·305 ml. of 0·1N per ml. of 0·1M-$NaIO_4$ gave pH 7·65). Precipitation never occurred with solutions (*b*) and (*c*) as here, but with (*b*) a precipitate appeared after a few days if all concentrations were doubled.

Consumption of periodate was measured by titration of 1- or 2-ml. samples with 0·005M-arsenite after addition of 4 ml. of 0·067M-phosphate of pH 7·5 and excess of potassium iodide. Polyvinyl alcohol containing 20 mol. % of residual acetyl (Miller and Bracken, *J.*, 1951, 1933) as indicator permitted titration to less than 0·005 ml. Volatile acid was determined as previously (Bell and Greville, *loc. cit.*). Formic acid was identified (1) by redistillation with mercuric sulphate (Bell and Greville, *loc. cit.*), (2) by testing evaporated neutralised distillate with chromotropic acid, before and after reduction with magnesium (Grant, *Analyt. Chem.*, 1948, **20**, 267), colours being compared by eye with standards.

Periodate consumed and acid formed are, unless otherwise indicated, expressed as moles (or equivs.) per mole of sugar initially present.

(*a*) *Reactions with metaperiodate.* (1) 2 : 3 : 6-Trimethyl glucose (9·5 mM), 2 : 4 : 6-trimethyl glucose (9·5 mM), and 2 : 3 : 4-trimethyl glucose (9·0 mM) were treated with 2·1 moles of metaperiodate :

Reaction time (hours)	8	12	25	50	100	200	480
Periodate consumed :							
2 : 3 : 6-Compound	0·00	—	0·01	0·01	0·03	0·06	—
2 : 4 : 6-Compound	0·00	—	0·00	0·01	0·02	0·04	—
2 : 3 : 4-Compound	0·02	0·06	0·15	0·27	0·46	0·78	1·25

2 : 3 : 4-Trimethyl glucose (11·1 mM) was also treated with 2·02 moles of metaperiodate for 12 hours. Periodate consumed : 0·10. Volatile acid formed : 0·00.

(2) (i) 3 : 4 : 6-Trimethyl glucose (*A*) and 3 : 4-dimethyl glucose (*B*) (each 9·0 mM), were treated with 2·13 moles of metaperiodate :

Reaction time (minutes)		5·7	10·5	20·7	42	63	85	135	480
Periodate consumed :	(*A*)	0·27	0·32	0·40	—	0·69	—	0·91	1·00
	(*B*)	0·59	0·62	0·67	0·76	—	0·88	—	1·00

(ii) 3 : 4 : 6-Trimethyl glucose (9·8 mM) was treated with 2·11 moles of metaperiodate at *ca.* 18°. Periodate consumed : 0·90 (3·2 hours), 0·99 (7·75 h.), 1·00 (24, 46, 70 h.), 1·01 (94 h.).

(iii) 3 : 4-Dimethyl glucose (9·2 mM) was treated with 2·08 moles of metaperiodate. Periodate consumed : 0·90 (1·5 hours), 0·99 (4 h.), 1·01 (7 and 53 h.), 1·04 (102 h.).

(3) 3 : 4 : 6-Trimethyl glucose (17·4 mM) was treated with 1·50 moles of metaperiodate :

Reaction time (hours)	0·58	20	45
Periodate consumed	0·65	1·00	1·01
Volatile acid formed	0·64	0·97	0·99

Redistillation with mercuric sulphate eliminated the volatile acid. The chromotropic test after reduction with magnesium gave about the expected intensity of colour; no formaldehyde was present before reduction and no formic acid in the control.

(4) To demonstrate the determination of 3 : 4 : 6-trimethyl glucose in the presence of the other trimethyl glucopyranoses, reaction mixtures (10 ml., containing 216 μmol. of metaperiodate) were set up as shown :

	Periodate consumed (μmols.)	Volatile acid formed (μequivs.)
(i) 101 μmol. 2 : 3 : 4-compound + 99 μmol. 2 : 3 : 6-compound + 105 μmol. 2 : 4 : 6-compound (12 hours)	10	0
(ii) As (i), + 101 μmol. 3 : 4 : 6-compound	108	102
(iii) Diff. due to 101 μmol. 3 : 4 : 6-compound	98	102
(iv) As (i), 60 hours	—	2

(5) 3 : 4-Dimethyl glucose (9·8 mM) was treated with 2·03 moles of metaperiodate for 12 hours. Periodate consumed, 1·00; volatile acid formed, 1·02.

(6) 2 : 3 : 4 : 6-Tetramethyl glucose (10·1 mM) was treated with 2·1 moles of metaperiodate. Periodate consumed : 0·00 (23, 51, 75 hours), 0·04 (141 h.), 0·12 (210 h.), 0·18 (289 h.), 0·28 (339 h.).

(7) 2 : 3 : 4 : 6-Tetramethyl β-methylglucoside (9·2 mM) was treated with 2·11 moles of metaperiodate. Periodate consumed : 0·00 (23 hours), 0·04 (142 h.), 0·13 (235 h.), 0·18 (281 h.), 0·26 (330 h.).

(8) 1 : 6-Anhydro-β-glucopyranose (9·0 mM) was treated with 3·01 moles of metaperiodate. Periodate consumed : 0·92 (1·13 hours), 1·43 (2·84 h.), 1·82 (6·67 h.), 2·01 (24, 53 h.). Acid present after 24 hours (by titration with sodium hydroxide after addition of excess of ethylene glycol, with methyl-red) : 1·00 equiv. per mole.

(9) 2-Methoxyethanol and *n*-butyl methyl ether (both 9·3 mM) were treated with 2·1 moles of metaperiodate. Periodate consumed : 0·00 (369 and 309 hours, respectively).

(*b*) *Reactions with periodate in phosphate*, pH 7·5. (10) 2 : 3 : 6-Trimethyl glucose (4·7 mM) was treated with 2·11 moles of periodate (Fig. 2), the sugar being in 2% solution in water for 40 hours before the reaction mixture was set up. An identical curve was obtained when the sugar was dissolved immediately before the start of the reaction. The compound (2·45 mM) was also treated with 4·06 moles of periodate. Periodate consumed : 0·56 (135 hours), 1·53 (267 h.), 2·96 (437 h.).

(11) 2 : 4 : 6-Trimethyl glucose (5·2 mM) was treated with 2·09 moles of periodate (Fig. 2). The sugar (2·35 mM) was also treated with 4·13 moles; periodate consumed : 0·25 (94 hours), 2·44 (219 h.), 4·13 (294 h.). As in (10), previous mutarotation had no effect on the shape of the curve.

(12) 2 : 3 : 4-Trimethyl glucose (4·7 mM) was treated with 2·08 moles of periodate (Fig. 1).

(13) (i) 3 : 4 : 6-Trimethyl glucose (9·0 mM) was treated with 2·11 moles of periodate at *ca.* 18° (cf. Expt. 2, ii). Periodate consumed : 1·04 (2·5 hours), 1·14 (9 h.), 1·24 (25 h.), 1·39 (47 h.), 1·55 (72 h.), 1·88 (125 h.).

(ii) 3 : 4 : 6-Trimethyl glucose (1·9 mM) was treated with 5·02 moles of periodate. Periodate consumed : 4·42 (336 hours).

(iii) 3 : 4-Dimethyl glucose (4·6 mM) was treated with 2·08 moles of periodate (cf. Expt. 2, iii). Periodate consumed : 0·91 (0·6 hour), 1·10 (7 h.), 1·45 (26 h.), 1·86 (55 h.), 2·03 (77 h.), 2·08 (102 h.).

(14) (i) 2 : 3 : 4 : 6-Tetramethyl glucose (5·2 mM) was treated with 2·09 moles of periodate. Periodate consumed : 0·04 (23 hours), 0·16 (47 h.), 0·36 (72 h.), 0·68 (99 h.), 1·02 (117 h.), 1·40 (135 h.), 1·83 (159 h.), 2·09 (192 h.). As in (10) previous mutarotation had a negligible effect on the shape of the curve.

(ii) The sugar (1·55 mM) was treated with 6·06 moles of periodate. Periodate consumed : 1·05 (99 hours), 6·06 (235 h.).

(iii) The sugar (0·40 mM) was treated with 24·2 moles of periodate. Periodate consumed : 1·0 (77 hours), 6·9 (125 h.), 10·8 (168 h.), 12·2 (215 h.), 13·6 (356 h.), 14·4 (478 h.), 14·5 (545, 601, 669 h.).

(iv) Volatile acid production and corresponding periodate consumption are shown :

Expt.	Sugar (mM)	Initial periodate	Periodate consumed	Volatile acid
(A)	1·6	6	2·7	0·9
(B)	1·6	6	6·0	1·2
(C)	0·4	24	14·6	0·3

With (B), redistillation with mercuric sulphate eliminated the volatile acid, and the chromotropic acid test gave the expected intensity of colour.

(15) (i) 3 : 4-Dimethyl β-methylglucoside (4·65 mM) was treated with 2·09 moles, 3 : 4 : 6-trimethyl β-methylglucoside (4·15 mM) with 2·33 moles, and 2 : 3 : 4 : 6-tetramethyl β-methylglucoside (4·6 mM) with 2·09 moles of periodate :

Time (hours)	50	100	150	200	250	300
Me_2	0·08	0·29	0·71	1·30	1·90	2·09
Me_3	0·12	0·62	1·40	2·01	2·33	—
Me_4	0·33	1·16	1·95	2·09	—	—

(ii) In addition, the tetramethyl glucoside (0·52 mM) was treated with 18·5 moles of periodate. Periodate consumed : 0·3 (30 hours), 4·7 (103 h.), 8·3 (168 h.), 12·2 (293 h.), 14·5 (500 h.), 15·1 (600 h.).

(iii) The tetramethyl glucoside (0·42 mM) was treated with 23·9 moles of periodate for 530 hours. Periodate consumed, 15·8; volatile acid produced, 0·3.

(16) The substances below did not consume any periodate :

Compound	Concn. (mM)	Periodate (moles)	Reaction time (hours)
2 : 3 : 4-Trimethyl 1 : 6-anhydro-β-glucose	5·0	2·1	291
2-Benzoyl 3 : 4 : 6-trimethyl β-methylglucoside	3·2	3·1	258
	1·3	7·7	258
2-Methoxyethanol	4·6	2·1	336
	1·5	6·3	213
n-Butyl methyl ether	4·6	2·1	309
	1·5	6·3	309

(17) Formic acid (" AnalaR "; 5 mM) was treated with 2·07 moles of periodate. Periodate consumed : 0·035 (29 hours), 0·05 (48 h.), 0·06 (208 h), 0·065 (308 h.).

(*c*) *Reactions with periodate* pH 7·6 *without addition of buffer.* (18) 2 : 3 : 4-Trimethyl glucose (5·6 mM) was treated with 2·08 moles of periodate (Fig. 1). pH values : initial, 7·63; final, 5·15.

(19) 2 : 3 : 6-Trimethyl glucose (4·65 mM) was treated with 2·09 moles of periodate and 2 : 4 : 6-trimethyl glucose (4·6 mM) with 2·13 moles (Fig. 3). pH values (16°) were measured at intervals. The control remained throughout at pH 7·64—7·66.

Periodate consumed	0·71	0·73	0·95	1·01	1·13	1·16	1·45
pH : 2 : 3 : 6-compound	—	7·06	6·86	—	6·58	—	—
2 : 4 : 6-compound	6·96	—	—	6·47	—	6·03	4·92

(20) 2 : 3 : 4 : 6-Tetramethyl glucose (4·55 mM) was treated with 2·10 moles of periodate (Fig. 3). Initial pH : 7·64.

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