327. *Oxidation of Some Simple Carbohydrates by Periodate in Phosphate and Other Buffers.*

By D. J. Bell, Anne Palmer, and (in part) A. T. Johns.

Periodate oxidation of certain carbohydrates has been examined in the presence of phosphate and other buffers. At pH 7·5, phosphate tends to promote " over-utilisation " of periodate, and the reactions proceed more rapidly than in carbon dioxide–bicarbonate or maleate buffers. It is therefore suggested that a complex ion may be formed between phosphate and periodate, and that the mode of oxidation by this complex differs from that by periodate itself.

In phosphate buffer (pH 7·5) 2 : 3 : 4-trimethyl glucose yielded considerably less than the expected one mole of formaldehyde. The " missing " formaldehyde was accounted for as formic acid. Increase in periodate concentration relative to phosphate favoured formaldehyde production, but this never approached the theoretical value. On the other hand, oxidation in phthalate (pH 6·2) was very slow, but finally produced one mole of formaldehyde and no formic acid. In contrast, the oxidation of 1 : 3 : 4-trimethyl fructose, in phosphate, took place "normally" to give one mole of formaldehyde.

3-Methyl glucose behaved "normally" in bicarbonate (7·5), but in phosphate (7·5) a low yield of formaldehyde resulted. 3 : 4 : 6-Trimethyl fructose, 3 : 4-dimethyl fructose, and fructose itself could not be made to yield the correct amounts of formaldehyde in a number of buffers and at different pH.

In phosphate, α-methylglucoside reduced considerably more than the theoretical two moles of periodate and the yield of formic acid tended to exceed somewhat the theoretical amount of one mole.

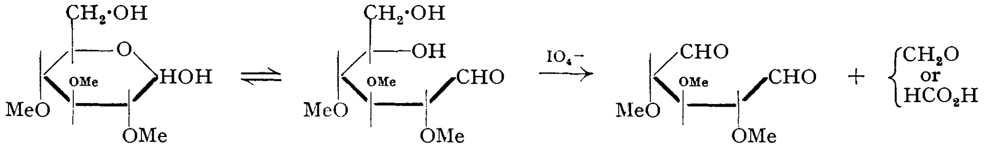
THIS paper extends observations made by one of us (Bell, *J.*, 1948, 992) when it was shown that the periodate ion, buffered to pH 7·5 by phosphate, reacted rapidly with certain, but not all, partly methylated sugars [each containing a ·CH(OH)·CH_2·OH group] examined, to yield the anticipated molar proportion of formaldehyde. The formaldehyde was determined and identified as the dimedon derivative. On the other hand, a number of the sugars reacted slowly, and in an "anomalous" manner in that the yield of formaldehyde was considerably less than that demanded by theory. The general procedure was, however, suitable for a small-scale qualitative test for the ·CH(OH)·CH_2·OH group in partly methylated sugars.

In order to facilitate certain researches we decided to investigate further several of these anomalous oxidations.

A notable example in the original work is provided by 2 : 3 : 4-trimethyl glucose. This substance exists only as a syrup and is known readily to part with the elements of water to form the 1 : 6-anhydro-derivative (Irvine and Oldham, *J.*, 1921, **119**, 1744). We therefore employed only material (prepared from crystalline 2 : 3 : 4-trimethyl 1 : 6-anhydroglucose) which had been freshly chromatographed on the silica–water partition column (Bell, *J.*, 1944, 473) and which, so far as could be ascertained, was analytically homogeneous.

We first sought to discover why the yield for formaldehyde from 2 : 3 : 4-trimethyl glucose was, in presence of phosphate, always below the theoretical, and also sought conditions under which the sugar could be induced to behave in the manner appropriate to its structure. We noted that previous workers had also failed to obtain theoretical yields of formaldehyde from 2 : 3 : 4-trimethyl glucose under a variety of conditions. Ariyama and Kitasato (*J. Biochem. Japan*, 1937, **25**, 357), using strongly acidified periodic acid, found only 0·02 mole of formaldehyde whilst Jeanloz (*Helv. Chim. Acta*, 1944, **27**, 1509) after 17-hours oxidation in carbon dioxide–bicarbonate buffer (containing sulphate) found 0·17 mole.

We confirmed our original observations that formaldehyde formation in phosphate buffer (pH 7·5) ceased long before the theoretical yield was attained. We examined the end-products of the reaction and found that a volatile acid having the properties of formic acid was produced in amounts which virtually accounted for the missing formaldehyde if it is assumed that only the primary alcohol group of the sugar was detached from the molecule :



Furthermore, the consumption of periodate in these experiments was considerably in excess of theoretical. Results comparable with those of the phosphate experiments were obtained using carbon dioxide–bicarbonate buffer at pH 7·5 [containing SO_4'' and prepared according to Jeanloz (*loc. cit.*)]. Price and Croll (*J. Amer. Chem. Soc.*, 1938, **60**, 2726) in the instance of pinacol, and both Reeves (*ibid.*, 1941, **63**, 1476) and Jeanloz in the instances of some carbohydrates, have emphasised the influence of pH on periodate oxidations of glycol groups. It seemed to us possible that two factors, at least, might operate in our anomalous oxidations. First, the effect of pH, and, secondly, an effect caused by complex-ion formation between periodate and phosphate in the one buffer, possibly between sulphate or carbonate in the other. If such a complex oxidant were in fact formed, its mode of attack on glycols might well pursue a course different from that of periodate alone. Thus, the effect of increasing the periodate concentration when phosphate was present was to increase the yield of formaldehyde with an exactly corresponding decrease in formic acid. On the other hand, oxidation in phthalate buffer (pH 6·2), where we did not expect complex formation, resulted in a slow liberation of formaldehyde, which, however, ultimately reached 97% of theory after 650 hours. Here, the theoretical amount of periodate was reduced and no volatile acid formed.

Our results are summarised in Table I, and the progress of the reactions is represented graphically in Fig. 1.

FIG. 1.

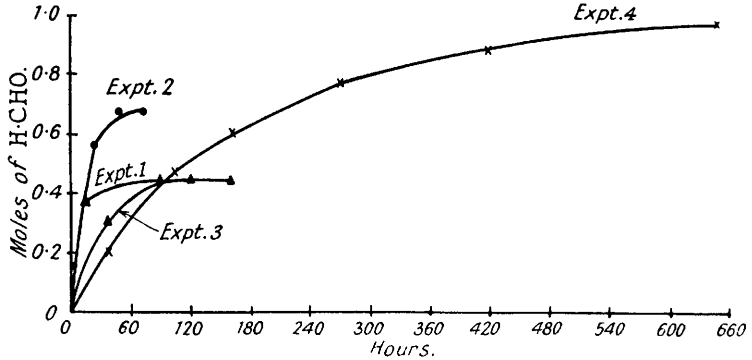


TABLE I.

Oxidation of 2 : 3 : 4-trimethylglucose.

Note.—In this and the following tables, molecular proportions are expressed in relation to 1 mole of substance oxidised. The columns " oxidation times " express, under " 1st " the time of the first measurement at which the reaction had reached a steady state, and under " 2nd " the time of the last measurement made, confirming completion of the oxidation. Results at " 1st " and " 2nd " times are identical.

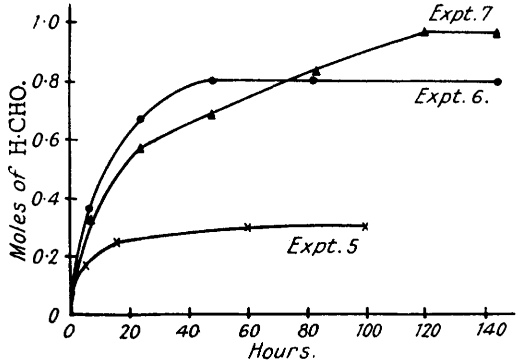
Serial No. of Expt.	Buffer.	pH.	Amount of IO_4^- (moles).	Oxidation times (hrs.).		Found.		
				1st.	2nd.	CH_2O (moles).	$H{\cdot}CO_2H$ (moles).	IO_4^- (moles) reduced.
1	Phosphate	7·5	4·1	90	120	0·44	0·55	4·1
2	,,	,,	9·3	48	72	0·66	0·27	—
3	Bicarbonate	,,	4·3	110	160	0·44	0·45	4·3
4	Phthalate	6·2	2·6	650	—	0·97	0·00	1·0

TABLE II.

Oxidation of 3-methyl glucose.

Serial No. of Expt.	Buffer.	pH.	Amount of IO_4^- (moles).	Oxidation times (hrs.).		Found CH_2O (moles).
				1st.	2nd.	
5	Phosphate	7·5	4	60	100	0·30
6	,,	,,	33	48	150	0·80
7	Bicarbonate	,,	33	120	150	0·97

FIG. 2.

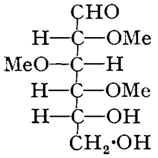


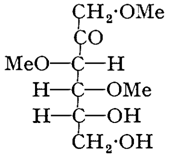
A second " anomalous " oxidation previously encountered was that of 3-methyl glucose. In acid periodate, Ariyama and Kitasato (*loc. cit.*) obtained 0·51 mole of formaldehyde. Bell

(*J.*, 1948, 992), using phosphate (pH 7·5) and four moles of IO_4^- per mole of sugar, found slow oxidation which ceased when about 0·25 mole of formaldehyde had been liberated. We have now found that, by use of a very large excess of periodate in bicarbonate buffer (pH 7·5) but not in phosphate, 0·97 mole of formaldehyde is formed. Results are summarised in Table II, and the courses of the reactions set forth in Fig. 2.

From experiments 5 and 6 it is again evident that pH is not the sole factor controlling formaldehyde formation. The suggestion of competitive oxidation indicated in experiments 1 and 2 is once more present.

A further point of interest is emphasised if the oxidation of 1 : 3 : 4-trimethyl fructose is compared with that of 2 : 3 : 4-trimethyl glucose. Theoretically, both sugars would be expected to behave in closely similar manner towards periodate since both have the same structure about carbons 3, 4, 5, and 6, when reacting in the open-chain form :


2 : 3 : 4-Trimethyl *aldehydo*-D-glucose.


1 : 3 : 4-Trimethyl *keto*-D-fructose.

1 : 3 : 4-Trimethyl fructose readily forms the theoretical one mole of formaldehyde in phosphate buffer, but, as in the case of 2 : 3 : 4-trimethyl glucose, over-consumption of periodate also takes place (Table III). This, however, is not so marked with the fructose compound. It is therefore possible, by formaldehyde determination, to detect and determine 1 : 3 : 4-trimethyl fructose in the presence of 1 : 3 : 4 : 6-tetramethyl fructose, since the latter does not react with periodate in phosphate unless contact with the reagents is prolonged beyond the time when formaldehyde liberation from the former is complete.

Experiments on the oxidation of 3 : 4 : 6-trimethyl fructose were also carried out. This sugar is known only as a syrup. Despite every care during the preparation of our material from trimethyl inulin, we are convinced that it is not completely homogeneous. Through the

TABLE III.

Oxidation of 1 : 3 : 4-*trimethyl fructose and* 1 : 3 : 4 : 6-*tetramethyl fructose in phosphate* (pH 7·5).

				Found.	
Serial No. of Expt.	Sugar.	Amount of IO_4^- (moles).	Oxidation times.	CH_2O (moles).	IO_4^- (moles) reduced.
8	Me_3	2·3	55	1·0	—
			70	1·0	—
9	,,	2·3	70	0·99	—
			120	0·99	—
10	,,	2·8	5	0·30	0·48
			24	0·80	1·28
			29	0·97	1·28
			48	1·01	1·50
			72	1·00	1·62
			96	1·00	1·81
11	Me_4	2·0	2	0	0
			22	0	0
			70	0·05	0·17
			94	0·09	0·30
			120	0·14	0·40

TABLE IV.

Oxidation of fructose (pH 7·5).

			Oxidation times (hrs.).		Found.	
Serial No. of Expt.	Buffer.	Amount of IO_4^- (moles).	1st.	2nd.	CH_2O (moles).	IO_4^- (moles) reduced.
12	Phosphate	6	3	96	1·68	4·6
13	,,	20	3	16	1·71	—
14	Maleate	6	24	48	1·74	4·1

TABLE V.

Oxidation of 3 : 4-*dimethyl fructose in phosphate* (pH 7·5).

Serial No. of Expt.	Amount of IO_4^- (moles).	Oxidation times (hrs.).	Found. CH_2O (moles).	Found. IO_4^- (moles) reduced.
15	4·4	29	1·72	1·78
		46	1·78	2·07
		70	1·78	2·21
		96	1·77	2·41

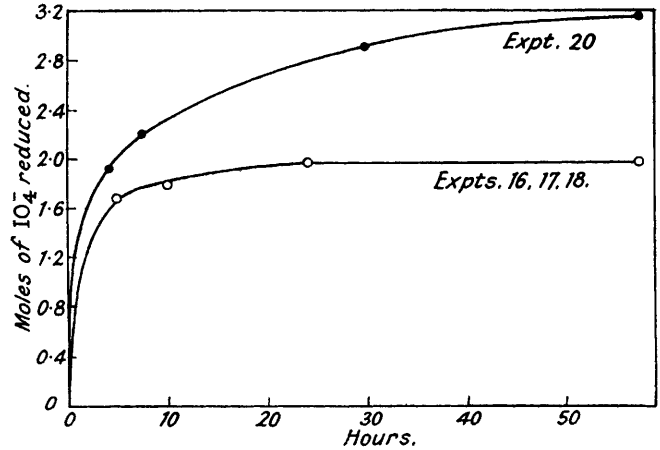
TABLE VI.

Oxidation of α-methylglucoside.

Serial No. of Expt.	Buffer.	pH.	Amount of IO_4^- (moles).	Oxidation times (hrs.). 1st.	Oxidation times (hrs.). 2nd.	Found. HCO_2H (moles).	Found. IO_4^- (moles) reduced.
16	Phthalate	6·2	2·8	72	96	—	1·98
17	,,	,,	3·5	48	78	1·0	2·0
18	Bicarbonate	7·5	3·5	24	48	1·1	2·0
19	Phosphate	,,	2·8	24	—	—	2·8
20	,,	,,	3·5	48	96	1·3	3·2

kindness of Dr J. K. N. Jones of Bristol University, an independent paper chromatogram was carried out by the elegant technique of Hirst, Hough, and Jones (this vol., p. 928). By this means a fast-running minor component was detected, in addition to the trimethyl sugar.

FIG. 3.



Similar results were obtained in this laboratory. From this evidence and other facts, one of which is recorded below, we believe that a proportion of 3 : 4 : 6-trimethyl fructofuranose when kept in the dry state, and also in aqueous solution, can undergo some degree of auto-condensation to form a non-reducing substance, probably a hexamethyl difructose anhydride (cf. Montgomery, *Adv. in Carb. Chem.*, 1946, **2**, 253). A freshly prepared sample of 3 : 4 : 6-trimethyl fructose yielded on periodate oxidation 0·9 mole of formaldehyde, but when the dry syrup or a solution in water was kept the formaldehyde yield fell to 0·80—0·82 moles on oxidation in phosphate. Oxidation at pH 7·5 in maleate or at 6·2 in phthalate gave similar results. There seemed to be a slight tendency to over-reduction of periodate. These findings are comparable with the results reported by other workers on the oxidation of fructose itself, *e.g.*, by Fleury and Lange (*Compt. rend.*, 1939, **209**, 219) using acid periodate, and both Reeves (*loc. cit.*) and Jeanloz (*loc. cit.*) using bicarbonate buffer at pH 7·5. Jeanloz also found that, although the yield of formaldehyde from fructose varied according to the pH, the theoretical value of 2 moles was never reached; results were, *e.g.*, at 7·5, 1·74 mole, at 8·8, 1·78 mole, and at 10·0, 1·22 mole. Our own results (cf. Jackson, "Organic Reactions," 1944, **2**, 341) on fructose at pH 7·5 are shown in Table IV.

Oxidation proceeded more rapidly in phosphate than in maleate buffer, without significantly altering the final yields of formaldehyde. The amount of periodate consumed in both buffers is less than the five moles expected.

Similar results were also obtained when 3 : 4-dimethyl fructose was investigated in phosphate buffer (Table V).

In conclusion, we append data relating to the oxidation of α-methylglucoside (Table VI and Fig. 3). In these examples it will be seen that use of phosphate buffer leads to marked over-utilisation of periodate.

EXPERIMENTAL.

Conditions used for oxidation, determination of formaldehyde and consumption of periodate were identical with those described by Bell (*J.*, 1948, 992). Blank experiments on formaldehyde determinations were found to be essential since the acetate buffer tends to decompose when kept, to yield a substance giving a precipitate with dimedon. Molarity of the buffers used for the oxidations varied from 0·066 to 0·2; investigation showed that, in all experiments, buffering was adequate.

In certain experiments, where the concentration of periodate was very high and where K^+ was present, aliquots of the solutions for future examination were pipetted into individual flasks, before any separation of potassium salts could take place.

Determination of Formic Acid (" *Volatile Acid* ").—The majority of these determinations were carried out by one of the authors as "unknowns." An aliquot of the reaction mixture (usually equivalent to about 5 mg. of carbohydrate) was treated with 2 ml. of pure ethylene glycol in order to destroy excess of periodate. After the solution had been kept for one hour, its pH was adjusted (red to thymol-blue, pH <1·2) by addition of saturated potassium hydrogen sulphate solution. The volume was then made up to 15 ml. A suitable aliquot (1 or 2 ml.) was pipetted into the distillation apparatus (Markham, *Biochem. J.*, 1942, **36**, 790), and 50 ml. of distillate were collected. This was titrated in a stream of CO_2-free air with CO_2-free 0·01N-NaOH using phenol-red as indicator. Experimental error was established as ±0·05%. A blank determination was simultaneously carried out on the reagents; this was of the order of 0·02 ml. of 0·01N-NaOH. The foregoing procedure is similar to that of Pirie (*Biochem. J.*, 1946, **40**, 100). Confirmation that the volatile acid was in fact formic acid was obtained by redistillation of the first distillate after boiling under reflux with mercuric sulphate (Friedemann, *J. Biol. Chem.*, 1938, **123**, 161). In experiments 1 and 3 on 2 : 3 : 4-trimethyl glucose, concentration of the distillates at pH 10 yielded material giving the formhydroxamic acid spot reaction.

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