The Periodate Oxidation of Methylfructoses.

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The extent of periodate oxidation of a number of methyl ethers of fructose has been measured by the determination of liberated formaldehyde and also by periodate uptake.

Periodate oxidation of methylated monosaccharides has been studied extensively by Bell (*J.*, 1948, 992), and Bell, Palmer, and Johns (*J.*, 1949, 1536). In the latter paper, the oxidation of 1 : 3 : 4- and 3 : 4 : 6-tri-*O*-methyl- and 3 : 4-di-*O*-methyl-D-fructose was described, and the liberated formaldehyde determined as its dimedone derivative. These workers found that a quantitative yield of formaldehyde was frequently difficult to obtain. Experiments on the molar uptake of periodate carried out concurrently gave much higher results than would be expected. This phenomenon was also observed by Hirst and Jones (*J.*, 1949, 1659) and Greville and Northcote (*J.*, 1952, 1945) during the oxidation of certain methylaldoses, and also by Neumüller and Vasseur (*Arkiv. för Kemi*, 1953, 235), while studying the action of periodate on disaccharides at different pH's.

In the present work the methyl ethers of fructose were oxidised at room temperature in the dark, and in view of the earlier anomalous results the formaldehyde obtained was determined both gravimetrically, by Bell's method (*loc. cit.*), and colorimetrically with chromotropic acid (see Eegriwe, *Z. analyt. Chem.*, 1937, **110**, 22); the quantity of periodate consumed was also measured. In order to determine the formaldehyde by the colorimetric method it is necessary first to destroy the excess of periodate, *e.g.* with stannous chloride (Corcoran and Page, *J. Biol. Chem.*, 1940, **146**, 279) or with sodium arsenite (Lambert and Neish, *Canad. J. Res., B.*, 1950, **28**, 83). The procedure finally adopted by the present authors was a modification of a method communicated by Mr. M. W. Rees of Cambridge (unpublished work), to whom they would like to record their thanks. The excess of periodate was removed by sodium hydrogen sulphite solution, and with each set of determinations a standard curve was constructed for glucose, the oxidation of which with liberation of formaldehyde takes place in a few minutes. This curve was linear up to a concentration of approximately 2 μg./ml. of solution. Although difficulties have been reported in the use of chromotropic acid we found good agreement between this method and the gravimetric method in experiments carried out on the same sample. The principal advantages of the colorimetric method are the speed of determination when several sugars are examined and the small amount of sugar, 200—500 μg., required for each estimation.

Although the yields of formaldehyde obtained from the methylated fructoses were reasonably high, yields of 100% were rarely obtained. The exceptions were occasional oxidations in bicarbonate buffer, but results in this medium were variable. The results for 3 : 4 : 6-tri-*O*-methyl- and for 3 : 4-di-*O*-methyl-D-fructose were in agreement with those recorded by Bell *et al.* (*loc. cit.*). The yield of formaldehyde from 1 : 3 : 4-tri-*O*-methylfructose by the colorimetric method was low and did not agree with the theoretical yield recorded by the above authors.

Fructose, 4-*O*-methyl-, 3 : 4-di-*O*-methyl-, and 3 : 4 : 6-tri-*O*-methyl-D-fructose gave nearly theoretical uptakes of periodate, but in every other derivative examined over-oxidation was encountered. With 1 : 4 : 5- and 1 : 4 : 6-tri-*O*-methylfructose the uptake of periodate after 24 hr. was extremely slow and did not exceed 1·4 mol. of periodate with either sugar. There does not appear to be any analogy with the results obtained by Northcote and Greville (*loc. cit.*) from similar methylated glucoses. The latter authors found for 2 : 4 : 6-tri-*O*-methylglucose a periodate consumption of 4·13 mol. of periodate during 294 hr. We consider that, under the mildly alkaline conditions of the experiment, partial enolisation may occur in these two methylated fructoses between $C_{(2)}$ and $C_{(3)}$, and that this is followed by attack by periodate.

TABLE 1. *Determination of formaldehyde gravimetrically.*

Sugar	Buffer	Duration of oxidn., hr.	H·CHO liberated, mol.	H·CHO expected, mol.
Fructose	Phosphate	48	1·70	2·0
	Bicarbonate	48	1·68	—
3 : 4 : 6-Tri-*O*-methylfructose	Phosphate	48	0·88	1·0
	,,	72	0·89	—
	No buffer	72	0·88	—
1 : 4 : 6-Tri-*O*-methylfructose	,,	72	0·10 *	0·0
	Phosphate	72	0·27	—
4-*O*-Methylfructose	No buffer	72	1·40	2·0
	Phosphate	72	1·38	—

* Impure precipitate—low m. p.

TABLE 2. *Determination of formaldehyde colorimetrically.*

Sugar	Buffer	H·CHO liberated, mol.				H·CHO expected, mol.
		Green filter		Yellow filter		
Fructose	Phosphate	1·6	1·7	—	1·72	2·0
	Bicarbonate	1·9	1·4	—	—	—
4-*O*-Methylfructose	Phosphate	1·66	1·76	—	1·70	2·0
	Bicarbonate	—	1·4	—	—	—
3 : 4-Di-*O*-methylfructose	Phosphate	1·58	1·62	—	1·76	2·0
	Bicarbonate	1·76	1·94	—	—	—
4 : 5-Di-*O*-methylfructose	Phosphate	0·83	0·85	0·83	0·90	1·0
	Bicarbonate	0·73	0·80	—	—	—
1 : 3 : 4-Tri-*O*-methylfructose	Phosphate	0·85	0·81	0·78	0·81	1·0
	Bicarbonate	0·79	0·82	—	—	—
3 : 4 : 6-Tri-*O*-methylfructose	Phosphate	0·92	0·89	0·84	0·92,0·90	1·0
	Bicarbonate	0·81	1·00	—	—	—

TABLE 3. *Molar consumption of periodate.*

Sugar	Duration of oxidn., hr.	Uptake, mol.	Expected uptake, mol.
Fructose	48	5·0	5·0
4-*O*-Methylfructose	48	2·7	3·0
	72	2·8	—
3 : 4-Di-*O*-methylfructose	48	2·0	2·0
4 : 5-Di-*O*-methylfructose	48	2·3	2·0
	72	2·5	—
1 : 3 : 4-Tri-*O*-methylfructose	48	1·6	1·0
	72	1·5	—
3 : 4 : 6-Tri-*O*-methylfructose	48	0·8	1·0
1 : 4 : 6-Tri-*O*-methylfructose	24	1·2	1·0
	48	0·9	—
	72	1·3	—
1 : 4 : 5-Tri-*O*-methylfructose	24	1·2	1·0
	48	1·3	—
	72	1·4	—
	168	1·4	—

EXPERIMENTAL

The buffer solutions (pH 7·5, verified in a pH meter) used in the determinations were molar sodium hydrogen carbonate (Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476), and 0·1M-citric acid (1·5 ml.) mixed with 0·2M-disodium hydrogen phosphate (18·5 ml.).

Estimation of Formaldehyde.—(a) *Gravimetric.* Bell's method (*loc. cit.*) was followed, a large excess of periodate being used, together with a crystallisation time of 18 hr.

(b) *Colorimetric.* A solution of the sugar (5 ml.) (sufficient to produce 50—100 μg. of formaldehyde) was mixed with phosphate buffer solution (0·5 ml.; pH 7·5) or with sodium hydrogen carbonate solution (M; 0·1 ml.), and sodium metaperiodate solution (0·3M; 0·1 ml.) in a tared boiling tube, and the stoppered mixture kept in the dark for 48 hr. Sodium hydrogen sulphite solution (s.g. 1·34; 0·2 ml.) was added to destroy excess of periodate, and, after shaking, chromotropic acid (4 : 5-dihydroxynaphthalene-2 : 7-disulphonic acid) (0·1M; 0·5 ml.) added. Concentrated sulphuric acid (20 ml.) was added slowly with cooling in a freezing-mixture. The colour was developed by heating the solution for 10 min. at 85°. After cooling, the volume of the solution was adjusted to 65 ml. Blank experiments were carried out in each determination.

The purple solution was examined in an absorptiometer, green (Ilford 604) and yellow (Ilford 606) filters being used. A calibration curve was constructed for each set of estimations, with glucose as a standard, and straight lines were obtained, with each filter, for quantities of formaldehyde from 10 to 120 μg.

Periodate Uptake.—The amount of periodate consumed was determined by Fleury and Lange's method (*J. Pharm. Chim.*, 1933, **17**, 107, 196). The oxidation was carried out in a bicarbonate buffer (1 g./10 ml. of solution).

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