359. *Quantitative Analysis of Mixture of Sugars by the Method of Partition Chromatography. Part III. Determination of the Sugars by Oxidation with Sodium Periodate.*

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Quantitative analysis, on the micro-scale, of reducing hexoses, pentoses, and methyl pentoses can be carried out by determining the formic acid produced when the sugars are oxidised with warm sodium periodate solution.

Determination, on the micro- or semi-micro-scale, of sugars separated on sheet filter paper by partition chromatography can be carried out by oxidation with Somogyi's copper reagent (*J. Biol. Chem.*, 1945, **160**, 61) or sodium hypoiodite, and the latter method can be used also for the micro-determination of the methylated aldose sugars (Jeanloz, *Helv. Chim. Acta*, 1946, **29**, 57; Hough, Hirst, and Jones, this vol., p. 928). Nevertheless, the use of these reagents suffers from disadvantages. For example, the copper reagent must be standardised against each individual sugar, whilst the alkaline hypoiodite reagent is not applicable to ketoses and does not oxidise quantitatively rhamnose and mannose. Sodium periodate oxidises compounds containing hydroxyl groups on adjacent carbon atoms with the formation of aldehydes and sodium iodate. If the resulting aldehydes possess a hydroxyl group on the carbon atom adjacent to the aldehyde group, further oxidation ensues with the formation of formic acid. For example, glycerol is converted quantitatively into two molecules of formaldehyde and one of formic acid on oxidation with periodates. The reducing hexose, pentose, and methyl pentose sugars contain hydroxyl groups on contiguous carbon atoms and would be expected to give formic acid on oxidation with sodium periodate, hexoses yielding five molecules, pentoses and methyl pentoses four molecules, and keto-hexoses three molecules of formic acid. Determination of the liberated formic acid is a relatively simple procedure, since it has been shown (Dunstan, Brown, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785; Halsall, Hirst, and Jones, *J.*, 1947, 1399) that, after addition of excess of ethylene glycol, the formic acid can be titrated in the normal manner, using methyl-red or screened methyl-red as indicator. Since oxidation of an aldose with alkaline hypoiodite leads to the consumption of only two equivalents of iodine, oxidation with sodium periodate should, other things being equal, give more accurate results and enable smaller quantities of sugars to be determined. The procedure outlined below is also quicker and simpler. It has been shown (Bell, *J.*, 1948, 992; Hughes and Nevell, *Trans. Faraday Soc.*, 1948, **44**, 941), that at ordinary temperatures the oxidation of the sugars and their methyl derivatives requires, in some cases, many hours for completion. To overcome this difficulty, oxidations were carried out on the boiling water-bath for 20 minutes, using a slight excess of 0·25M-sodium periodate. If too great an excess of the reagent is used, undue loss of formic acid may occur, and on the other hand, if too little is used, the reaction is not completed in 20 minutes. As a result of many experiments, it was found that 1 c.c. of 0·25M-sodium periodate was sufficient to oxidise, in 20 minutes, amounts of sugar ranging from 0·08 to 3·9 mg. in 6 c.c. of solution (see Tables I and III for results). Subsequent oxidation of the formic acid and of the formaldehyde produced in the reaction occurs to a small extent under the conditions employed but, even so, the yield of formic acid is about 96%. Sorbose gives low yields of acid and requires oxidation for a longer time before a 96% yield of formic acid results. Under the conditions used, the amount of formic acid produced from the liberated formaldehyde is so small that it may be neglected.

The oxidation of several derivatives of the simple sugars was also examined. Galacturonic acid yielded 96% of the expected amount of formic acid (five molecules) in 20 minutes, and the carbon dioxide produced in the reaction did not interfere with the determination. Glucosamine hydrochloride on oxidation should yield five molecules of formic acid, one of formaldehyde, and one of ammonium chloride, which could react with the formaldehyde yielding hexamine and might therefore be expected to lead to ambiguous results; in fact, however, 99% of the theoretical yield of acid was produced. Inositol behaved abnormally, yielding a low but consistent value of 75% of the expected quantity of acid (6 molecules); anomalous results with inositol have been encountered by other workers (Fleury, Poirot, and Fievet, *Compt. rend.*, 1945, **220**, 664).

Oxidation of partly methylated sugar derivatives gave ambiguous results, in some case more, and in others less, than the theoretical quantities of acid being produced. For example, 3 : 4-dimethyl rhamnose, which was expected to yield one molecule of acid, gave a 35% yield,

whilst 2 : 3-dimethyl glucose gave two molecules of formic acid when only one was to be expected (cf. Bell, *J.*, 1948, 992) The method is thus not satisfactory for the determination of methylated sugars.

As an example of the application of the method, a mixture of rhamnose, ribose, arabinose, and galactose was separated on the paper chromatogram and the sugars were determined by the method described above. Recoveries of 106—102% of the amounts actually present in the original mixture were achieved, when ribose is used as the reference sugar.

TABLE I.

2 C.c. of 0·25M-sodium periodate. Total vol., 7 c.c.

Sugar.	Duration of oxidation (mins.).	Amount of sugar taken (mg.).	Yield of formic acid. mg.	mols./mol.	%
Ribose	20	0·795	0·915	3·76	94
	40	0·795	0·935	3·84	96
	60	0·795	0·895	3·68	92
Arabinose	15	0·548	0·633	3·80	95
	15	0·822	0·950	3·80	95
	15	1·370	1·562	3·76	94
	30	0·274	0·312	3·76	94
	30	1·370	1·580	3·80	95
	20	0·086	0·0915	3·48	87
	20	0·259	0·296	3·72	93
	20	0·431	0·517	3·92	98
	40	0·086	0·096	3·64	91
	40	0·259	0·302	3·80	95
	40	0·431	0·491	3·72	93
Xylose	5	0·589	0·476	2·64	66
	20, 40, & 60	0·589	0·722	4·00	100
Rhamnose hydrate	10	0·738	0·708	3·80	95
	20	0·738	0·737	3·96	99
	30	0·738	0·715	3·84	96
	40	0·738	0·693	3·72	93
	20	1·320	1·268	3·84	96
Glucose	10	0·715	0·836	4·60	92
	20	0·715	0·831	4·60	92
	40	0·715	0·869	4·80	96
Galactose	10	0·725	0·792	4·30	86
	20	0·725	0·844	4·55	91
	40	0·725	0·895	4·80	96
	60	0·725	0·860	4·65	93
Mannose	20 & 40	1·00	1·214	4·80	96
Fructose	20, 40, & 60	0·895	0·653	2·85	95
Sorbose	10	0·463	0·315	2·64	88
	20	0·463	0·346	2·91	97
	40	0·463	0·346	2·91	97
	60	0·463	0·323	2·70	90
Galacturonic acid	20	0·741	0·846	4·80	96
	40	0·741	0·731	4·40	88
	60	0·741	0·621	3·50	70
Glucosamine hydrochloride	20	1·10	1·158	4·95	99
	40	1·10	1·038	4·45	89
	60	1·10	0·975	4·25	85
Sucrose	29, 40, & 60	0·487	0·0645	0·99	99
Inositol	20	0·416	0·488	4·50	75
	40	0·416	0·459	4·32	72
	60	0·416	0·484	4·56	76

TABLE II.

Oxidation of methylated sugars.

2 C.c. of 0·25M-sodium periodate. Total vol., 7 c.c.

Sugar.	Duration of oxidation (mins.).	Amount of sugar taken (mg.).	Yield of formic acid. mg.	Yield of formic acid. mols./mol.
6-Methyl glucose	20	0·99	0·861	3·60
	40	0·99	0·833	3·52
	60	0·99	0·766	3·24
3-Methyl glucose	20	0·672	0·242	1·52
	40	0·672	0·318	2·00
	60	0·672	0·318	2·00
2 : 6-Dimethyl galactose	20	0·662	0·233	1·52
	40 & 60	0·662	0·287	1·96
2 : 3-Dimethyl glucose	20	1·172	0·560	2·16
	40	1·172	0·545	2·10
	60	0·586	0·267	2·06
4 : 6-Dimethyl glucose	20	1·018	0·504, 0·517	2·24, 2·30
	40	1·018	0·575, 0·575	2·56, 2·56
	60	1·018	0·585, 0·607	2·60, 2·70
3 : 4-Dimethyl rhamnose	20	0·802	0·067	0·35
	40	0·802	0·100	0·52
	60	0·802	0·117	0·61

TABLE III.

1 C.c. of 0·25M-sodium periodate. Duration of oxidation, 20 mins. Total vol., 6 c.c.

Sugar.	mg.	Yield of formic acid. mg.	Yield of formic acid. mols./mol.	Yield of formic acid. %
Ribose	0·274	0·321	3·84	96
	1·369	1·545	3·68	92
Arabinose	0·086	0·157	4·04	101
	0·431	0·512	3·92	98
Xylose	0·201	0·252	4·08	102
	1·205	1·410	3·84	96
Rhamnose hydrate	0·156	0·157	3·96	99
	0·782	0·786	3·96	99
	3·91	3·93	3·96	99
Glucose	0·251	0·317	4·95	99
	1·254	1·535	4·80	96
Mannose	0·211	0·262	4·85	97
	1·06	1·355	4·75	95
Galactose	0·244	0·258, 0·266 *	4·50, 4·65 *	90, 93 *
	1·12	1·33, 1·336 *	4·65, 4·70 *	93, 94 *
Fructose	0·225	0·162	2·82	94
	1·123	0·780	2·70	90
Sorbose	0·195	0·146, 0·156 *	2·94, 3·00 *	98, 100 *
	0·976	0·594, 0·588 *	2·40, 2·37 *	80, 79 *
Dulcitol	0·211	0·210	3·96	99
	1·053	1·061	3·96	99

* After 40 minutes' oxidation.

EXPERIMENTAL.

General Procedure.—Reactions were carried out in test-tubes, 22 × 3 cm., fitted with a B-34 ground joint and stopper. The apparatus was cleaned with chromic acid and well washed with distilled water before use.

Sodium metaperiodate was prepared from sodium paraperiodate by recrystallisation from nitric acid. It contained no free acid since, on addition of excess of ethylene glycol to its aqueous solution, sodium iodate neutral to methyl-red was formed. The ethylene glycol was neutral to methyl-red.

The sugar (0·2—3 mg.) was dissolved in water (*ca.* 5 c.c.) and oxidised with *ca.* 0·25M-sodium metaperiodate on the boiling water-bath for the requisite length of time. The solution was then cooled under the tap, and ethylene glycol (*ca.* 0·2 c.c.) added to destroy excess of periodate. The formic acid was titrated with 0·01N-sodium hydroxide, using methyl-red or screened methyl-red as indicator. At the end-point of the titration, the colour of the solution was identical with that of a control solution containing ethylene glycol and indicator. Under these conditions formic acid is slowly destroyed, and formaldehyde is slowly oxidised to formic acid, but neither reaction is sufficiently rapid to invalidate the method (see below).

Control Experiments with Formaldehyde.—An aqueous solution of ethylene glycol (0·005M.; 5 parts) was oxidised with sodium metaperiodate solution (2 parts) on the boiling water-bath. This gave a solution 0·01M. with respect to formaldehyde. At intervals, samples were cooled under the tap, excess of glycol was added, and the formic acid was titrated. Found, c.c. of 0·01N-acid per 5 c.c. of ethylene glycol solution: 0·15 (20 minutes), 0·38 (40 minutes), 0·63 (1 hour).

Control Experiments with Formic Acid.—Portions of 0·01N-formic acid (5 c.c.) were oxidised with sodium metaperiodate (2 c.c.) on the boiling water-bath for the requisite length of time. The sample was cooled, the periodate was destroyed, and the formic acid was titrated. Found: c.c. of 0·01N-formic acid: 4·9 (20 minutes), 4·7 (40 minutes), 4·7 (1 hour).

In a second experiment, samples containing 0·01N-formic acid (1 c.c.), water (4 c.c.), and sodium metaperiodate (2 c.c.) were heated on the boiling water bath. Found: c.c. of 0·01N-formic acid: 0·94 (20 minutes), 0·91 (40 minutes), 0·84 (1 hour).

The experiments were repeated using less sodium periodate solution (1 c.c.) in order to lower the rates of oxidation of formaldehyde and formic acid.

Control Experiments with Formaldehyde.—Solutions of ethylene gycol (0·005M) were oxidised with sodium metaperiodate (1 c.c.), yielding solutions of formaldehyde (0·01N.) which were then heated for the requisite time. Found, c.c. 0·01N-acid produced: (*a*) 0·01 c.c. from 1 c.c. of solution, diluted with water (4 c.c.) after 20 minutes and 40 minutes. (*b*) 0·10 c.c. from 5 c.c. of solution after 20 minutes and 40 minutes.

Control Experiments with Formic Acid.—Formic acid (5 c.c., 0·01N.) was heated with sodium metaperiodate (1 c.c.) for the requisite time. Found, % recovery of formic acid: 1 c.c. of formic acid solution gave 95% and 94% yield after 20 and 40 minutes, respectively; 5 c.c. of formic acid solution gave 96% and 92% yield after 20 and 40 minutes, respectively. These experiments show that the recovery of formic acid is not dependent on its concentration.

Separation and Determination of a Mixture of Ribose, Arabinose, Galactose, and Rhamnose.—A mixture of ribose (18·47 mg.), arabinose (20·48 mg.), galactose (24·02 mg.), and rhamnose (36·50 mg.) was dissolved in water (2 c.c.), and the sugars were separated on the paper chromatogram by the method of Flood, Hirst, and Jones (*J.*, 1948, 1681). The sugars were extracted from the paper strips by placing the paper in the cup of a small Soxhlet apparatus, fitted with B-34 ground joints and of the type described by A. A. Morton ("Laboratory Technique," p. 202, McGraw-Hill Book Co. Inc., 1938). The blank due to the paper was never more than 0·10 c.c. of 0·01N-sodium hydroxide (Found: 0·07, 0·07, 0·05, 0·10, 0·06, 0·07). The sugars, thus obtained as solutions in water (*ca.* 5 c.c.), were oxidised with sodium metaperiodate solution (1 c.c.) on the boiling water-bath for 20 minutes. The solutions were then cooled, and the excess of sodium periodate was destroyed by the addition of ethylene glycol. Methyl-red (2 drops) was added and the formic acid titrated with 0·01N-sodium hydroxide. Found: ribose, 0·176 mg.; arabinose, 0·206 mg.; galactose, 0·241 mg.; rhamnose 0·355 mg. The amounts of arabinose, galactose, and rhamnose calculated to have been present in the original mixture are, therefore, 21·6 mg. (106% recovery), 25·3 mg. (104% recovery), and 37·2 mg. (102% recovery), respectively, ribose being used as the reference sugar.

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