358. *Stability of the Glycosidic Linkages in Carbohydrates.*

By WINIFRED G. WRIGHT.

Lactose, cellobiose, and turanose are almost completely oxidised by periodic acid in 24 hours, with fission of the glycosidic linkage, whereas sodium or potassium periodate attacks only the α-glycollic functions in that time.

A method of detecting the degree of hydrolysis of carbohydrates with mineral acids is indicated, applicable also to keto-sugars, where the reducing groups cannot be estimated in the usual manner with hypoiodite.

COURTOIS and RAMET [1] found lactose to be completely oxidised by periodic acid within four da ys at room temperature, according to the equation :

$$C_{12}H_{22}O_{11} + 11O = 2CH_2O + 9H{\cdot}CO_2H + CO_2$$

Earlier stages in the oxidation were not recorded. Courtois, Wickstrom, and Le Dizet [2] found that at 0—2° only 8·65 atoms of oxygen were consumed in 15 days. We have found that at room temperature 8·46 equivalents of acid are produced within 24 hours on oxidation with periodic acid, whereas only 2·28 equivs. are produced by the potassium salt (see Table 1 and Fig 3).

Head and Hughes [3] record that with 0·15M-sodium metaperiodate at 20°, cellobiose absorbs five oxygen atoms in 24 hours, and eleven atoms within 50 days. At the same temperature we have found an absorption of ten atoms of oxygen, with the production of 8·56 equivalents of acid, within 23 hours, when the oxidising agent was periodic acid, and an absorption of only 4·67 atoms when it was the potassium salt (0·016M) (Table 3, Fig. 1).

On oxidation with potassium periodate, turanose absorbed 5·5 atoms of oxygen in 48 hours, only the α-glycollic group being attacked in that time, whereas in 24 hours, with periodic acid, 8·36 atoms of oxygen were absorbed with the production of seven equivalents of acid (Fig. 2, Table 4).

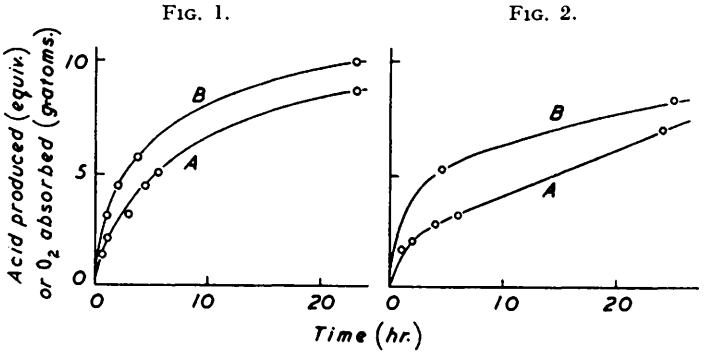
[1] Courtois and Ramet, *Bull. Soc. Chim. biol.*, 1947, **29**, 240.
[2] Courtois, Wickstrom, and Le Dizet, *ibid.*, 1952, **34**, 1121.
[3] Head and Hughes, *J.*, 1954, 603.

TABLE 1. *Oxidation of lactose.*

Oxidant	Lactose concn. (M)	Time (min.)	Acid produced (equiv.)	After 5 min. in 3% H_2SO_4 at 100° Oxidant	Lactose concn. (M)	Time (min.)	Acid produced (equivs.)
HIO_4, 0·1082N ...	0·00746	50	2·15	HIO_4, 0·1120N ...	0·00197	60	2·25
		80	2·43			75	2·45
		110	2·70			90	2·71
0·1118N ...	0·00761	180	3·30			112	2·80
0·1541N ...	0·00660	270	4·94	KIO_4, 0·0158N ...	0·00325	65	1·31
		390	6·04			120	1·68
		1330	8·46			180	1·98
$NaIO_4$, 0·0733N	0·00593	60	1·67			240	2·05
		90	1·82			300	2·17
		105	1·86			1500	2·28
		128	1·96				
		143	2·03				
		162	2·01				
		177	2·01				

In all these cases, overoxidation is exceedingly slow (negligible in 24 hours) when the salts of periodic acid are used, whereas almost complete oxidation takes place in that time when the free acid is used.

FIG. 1. FIG. 2.



A, *Acid produced* (*equivs.*). *B*, O_2 *absorbed* (*atoms*).

FIG. 1. *Oxidation of cellobiose with* 0·1027N-*periodic acid.*

FIG. 2. *Oxidation of turanose with* 0·1216N-*periodic acid.*

Oxidation for 24 hours with the potassium salt, and then with the free acid, should give an indication of the presence of 1 : 3- and 1 : 4-bonds in polysaccharides.

The course of the oxidation of turanose is shown in the annexed scheme. The first stage is formation of a formic ester, hydrolysis and subsequent oxidation of which gives rise [4] to the active hydrogen atom, marked *. By stage 6, the glycosidic linkage of the saccharide has become part of a carbonic ester and is then hydrolysed. Eleven oxygen atoms in all are absorbed, with the production of three mols. of formaldehyde, seven of formic acid, and two of carbon dioxide. Essentially the same reaction mechanism would apply to the keto- or pyranose form of the sugar.

The 1 : 4-glycosidic linkage of lactose was not hydrolysed by boiling 3% mineral acid in five minutes, curves representing the course of oxidation with periodic acid before and after treatment with the mineral acid being coincidental (Fig. 3, curve *B*).

Similar results were obtained with raffinose (Fig. 4). The 1 : 2-bond was hydrolysed by boiling 3% mineral acid in five minutes, and, after neutralisation, the mixture was

[4] Sprinson and Chargaff, *J. Biol. Chem.*, 1946, **164**, 433; Huebner, Ames, and Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621.

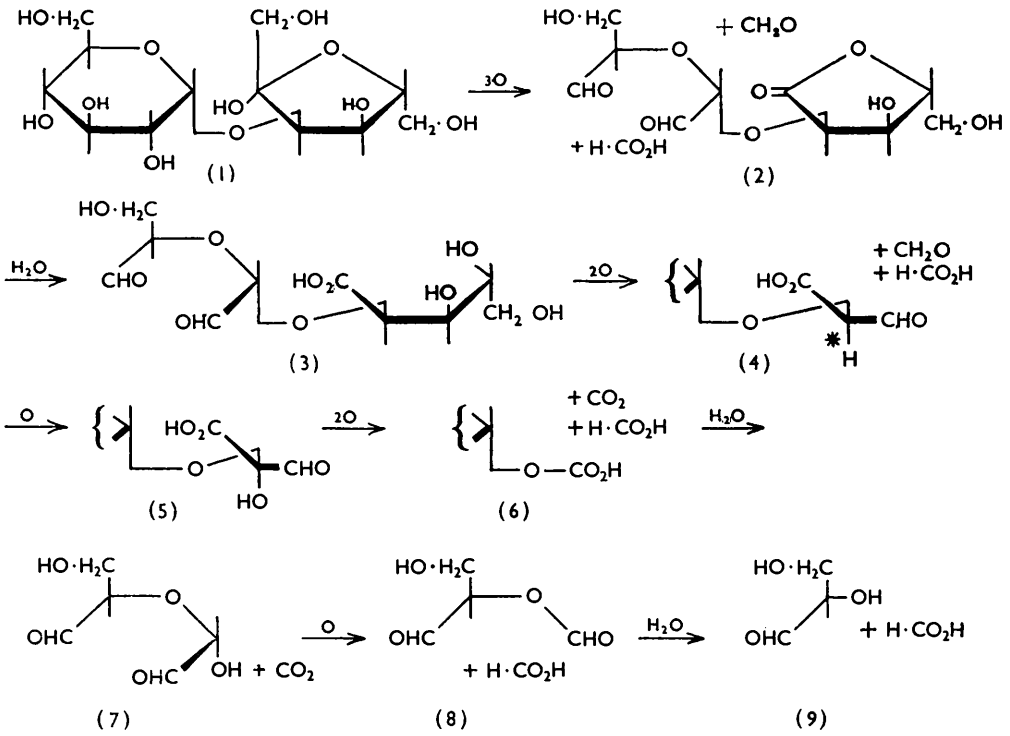


TABLE 2. *Oxidation of raffinose after 5 minutes' hydrolysis by 3% sulphuric acid at 100°*

Reactants	Time (min.)	O_2 abs. (atoms)	Acid produced (equiv.)	Reactants	Time (min.)	Acid produced (equiv.)
	60	—	4·08			
KIO_4, 0·0136N ...	240	—	5·42	HIO_4, 0·1120N	75	5·19
Sugar, 0·00125M	300	—	5·62	Sugar, 0·00118M	90	5·38
	360	8·25	6·02		130	5·62
$NaIO_4$, 0·0473N	120	8·05	—		180	6·13
Sugar, 0·00109M				$NaIO_4$, 0·0733N	150	6·11
				Sugar, 0·00071M		

Oxidation of raffinose after 24 hours' hydrolysis by cold 10% sulphuric acid.

Reactants	Time (min.)	O_2 abs. (atoms)	Acid produced (equiv.)
KIO_4, 0·0121N	45	—	3·97
	75	—	4·55
Sugar, 0·00095M	95	—	4·62
	135	7·43	—
	173	—	5·20
	233	—	5·39
	240	7·93	—
	420	8·40	5·95

TABLE 3. *Oxidation of cellobiose with 0·1027N-periodic acid.*

Cellobiose concn. (M)	Time (hr.)	Acid produced (equiv.)	Cellobiose concn. (M)	Time (hr.)	O_2 abs. (atoms)	Acid produced (equiv.)
0·00840	0·5	1·40	0·00786	3·75	5·72	—
	1	2·05		4·5	—	4·41
	3	3·17		5·5	—	5·00
				23	9·98	8·56

oxidised with potassium periodate. After treatment for 24 hours with cold 10% sulphuric acid, oxidation with potassium periodate gave points on the same curve, proving that the 1 : 6-bond is stable in the stronger acid.

TABLE 4. *Oxidation of* 0·00526M-*turanose with* 0·1027N-*periodic acid.*

Time (hr.)	1	2	4	4·5	6	24	25
O_2 absorbed (atoms)	—	—	—	5·29	—	—	8·36
Acid produced (equiv.)	1·72	2·11	2·80	—	3·30	7·03	—

Any displacement of such curves should give a measure of hydrolysis, and should be particularly useful in the examination of polysaccharides containing keto-sugars, where the reducing groups cannot be estimated by means of hypoiodite.

FIG. 3. FIG. 4.

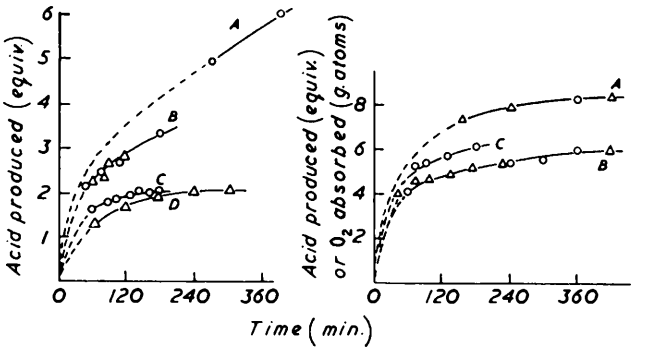


FIG. 3. *Oxidation of lactose.*

A, 0·1541N-HIO_4. *B*, *Points* ○, 0·1541N-HIO_4; *points* △, 0·1120N-HIO_4 *after* 3 *minutes in* 3% H_2SO_4 *at* 100°. *C*, 0·0733N-$NaIO_4$. *D*, 0·0158N-KIO_4 *after* 3 *minutes in* 3% H_2SO_4 *at* 100°.

FIG. 4. *Oxidation of hydrolysed raffinose.*

A, O_2 *absorbed* (*atoms*) *with* KIO_4. *B*, *Acid produced with* KIO_4. *In A and B*; *points* ○, *after hydrolysis with* 3% H_2SO_4, △, *after hydrolysis with* 10% H_2SO_4. *C*, *Acid produced with* HIO_4.

EXPERIMENTAL

The solutions of saccharides and periodate, or periodic acid, were kept in the dark, at constant temperature, samples being withdrawn at intervals, and. after destruction of the excess of periodic acid or periodate with ethylene glycol, were titrated with standard sodium hydroxide, bromothymol-blue being used as an indicator.

Absorption of oxygen was determined by the addition of sulphuric acid and potassium iodide, and titration of the liberated iodine, in the usual manner, with thiosulphate.

When the sugars were treated with mineral acid, the solutions were neutralised with sodium hydroxide before addition of the oxidising agent.

The results are recorded in the Tables and Figures.

The conditions of hydrolysis of lactose (3% acid, 100°, 5 min.) were determined as follows. Treatment of lactose with sodium hypoiodite gave a value of 0·993 CHO group; this was increased only to 1·03 by 10 minutes' previous boiling with 3% sulphuric acid, indicating absence of hydrolysis.

Similarly, after being boiled with 3% sulphuric acid for 5 minutes, raffinose gave, on treatment with hypoiodite, a value of 1·01 CHO groups, indicating fission of the sucrose bond only. After 10 minutes' boiling the value rose to 1·21, and after 30 minutes' to 1·38. One hour's boiling with 10% acid gave a figure of 2·12.

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