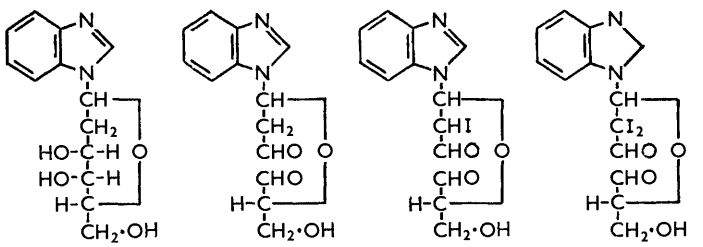
513. *Periodate Oxidation of Deoxy-sugar Derivatives.*

By A. J. Cleaver, A. B. Foster, E. J. Hedgley, and W. G. Overend.

There is an apparent high uptake of periodate by deoxy-sugar derivatives when the determination is made by titrating with sodium arsenite the iodine liberated from excess of periodate. Iodination of the methylene group in the oxidation products has been shown to occur during the determination.

Recently syntheses have been described of 2-(polyhydroxyalkyl)benzimidazoles [1] and 1-glycosylbenzimidazoles [2] derived from 2-deoxy-sugars. Condensation in xylene of tri-*O*-acetyl-2-deoxy-D-galactosyl bromide and benzimidazolylsilver afforded, after deacetylation, a lævorotatory form (II) of 1-2′-deoxy-D-galactosylbenzimidazole whereas condensation in dioxan yielded, not only this compound, but also a larger quantity of a dextrorotatory isomer (I). It is probable that these compounds are α- and β-pyranose forms (a pyranose ring is assumed in formulæ I and II) but this has not been proved and so attempts have been made by periodate oxidation to provide information about the ring size in the sugar moiety. Anomalous results were obtained in the case of isomer (II). Rather than the expected amount (1 mol.), compound (II) was found to consume 1·47 mol. of periodate



(I) (+)- (III) (IV) (V)

(II) (-)-

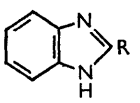
when the uptake of oxidant was determined by iodometric titration with sodium arsenite. When the neutralised oxidation mixture was stored after addition of potassium iodide, the iodine liberated from the unused periodate gradually disappeared. Polarimetric observation indicated that periodate oxidation of isomer (II) was complete within 2 minutes and a colourless crystalline substance was then obtained which gave positive tests with aldehyde reagents. A solid picrate of this compound gave analyses correct for the compound expected from substance (III). [Although formulæ (III), (IV), and (V) represent carbonyl compounds, the compounds are probably internal hemialdals.] This indicates that (*a*) 1 mol. of sodium periodate was consumed in the initial oxidation of the glycosyl derivative (II), and (*b*) the compound possessed a pyranose ring since the corresponding furanose derivative would have been oxidised with the loss of $C_{(6)}$ of the sugar moiety and the formation of a picrate with a nitrogen content greater than was found.

As the arsenite method of determination of the periodate consumed by 1-β-D-galactopyranosylbenzimidazole gave the expected results (2 mol. of oxidant consumed and 1 mol. of formic acid liberated) it appeared that the anomalous behaviour of compound (II) was associated with the 2-deoxy-group. In the oxidation product (III) this methylene group will be " active " and will react with iodine liberated from the excess of periodate, leading to compounds of types (IV) and (V). Removal of iodine from solution in this way will result in a low titre in the determination and an apparently high uptake of periodate by the

[1] Cleaver, Foster, and Overend, *J.*, 1957, 3961.
[2] *Idem*, *J.*, 1959, 409.

glycoside (II). This possibility was investigated by treating a solution of the benzimidazole derivative (II) with an exact quantity of sodium periodate to produce, after treatment with sodium hydrogen carbonate and potassium iodide, an equimolecular mixture of product (III) and iodine. Gradually the iodine colour faded and a cream-coloured solid was deposited which gave a correct elemental analysis for a compound (IV). Of several possible structures for the monoiodo-compound, (IV) seems the most probable. When a further mol. of solid iodine was added to the mother-liquors, the brown colour was again discharged, indicating further iodination, possibly leading to a compound of type (V). When reaction between the aldehyde (III) and iodine was followed titrimetrically, rapid consumption of between 2 and 3 mol. of iodine was noted, indicating the formation possibly of derivatives more highly iodinated than (V).

When 2-(D-*lyxo*-2 : 3 : 4 : 5-tetrahydroxypentyl)benzimidazole was oxidised by periodate it apparently consumed 5 mol. of oxidant (expected 3 mol.) (the method of determination was essentially that of Huebner *et al.*[3]) and liberated 1·7 mol. of formic acid. Similarly 2-(D-*threo*- and 2-(L-*erythro*-2 : 3 : 4-trihydroxybutyl)benzimidazole each consumed 2 mol. more oxidant than expected when the determination was carried out iodometrically with sodium arsenite. The uptake of periodate was complete within 5 minutes. Initial oxidation of each compound leads to 2-benzimidazolylacetaldehyde (VI) which might be expected to react with iodine liberated from excess of periodate. This would lead to a higher uptake than expected of periodate when the determination is based on an iodometric titration. An alternative scheme analogous to that found by Huebner *et al.*[3] to occur in the oxidation of 2-(1 : 4-anhydrotetrahydroxybutyl)benzimidazoles is considered to be less likely. This scheme involves oxidation of (VI) to (VII) which undergoes further periodate cleavage with the production of 2-formylbenzimidazole and an additional mol. of formic acid: in fact this extra formic acid was not detected.



(VI) R = CH_2CHO

(VII) R = CH(OH)CHO

In view of these results the periodate oxidation of some simple 2-deoxy-sugar derivatives and 2-deoxyhexitols was examined. In each case the excess of periodate was determined by addition of potassium iodide to the neutralised solution and direct estimation of the iodine produced by standard sodium arsenite. Results are shown in the Table: in each case there is an apparent consumption of oxidant in excess of that expected

Periodate oxidation of derivatives of 2-deoxy-sugars. Mean consumption of $NaIO_4$ *plus* I_2 *(mols./mol. of derivative).*

Storage time after iodine release	0—2 min.	2—3 min.	0·5—1 hr.	2—5 hr.	24 hr.	3 days	Limit of error (±)
Me 2-deoxy-α-D-glucoside	1·24	1·54	2·42	2·58	3·08	3·15	0·04
Me 2-deoxy-α-D-galactoside	—	—	—	—	3·03	—	0·04
Me 2-deoxy-β-L-riboside	1·0	—	1·78	—	2·26	2·81	0·07
p-Chlorophenyl 2-deoxy-α-D-glucoside	—	2·21	—	5·84	>6·2	—	0·05
p-Tolyl 2-deoxy-α-D-glucoside	—	—	—	—	7·50	—	0·1
2-Deoxy-D-glucitol	—	3·13	—	—	5·76	—	0·03
2-Deoxy-D-galactitol	—	3·06	—	—	>5·0	—	0·02
p-Chlorophenyl 3 : 4 : 6-tri-*O*-acetyl-2-deoxy-α-D-glucoside	0	—	—	—	0	—	0·2

for normal glycol cleavage. There is an inverse correlation between the iodine titre and the time interval between liberation and estimation of the halogen. That *p*-chlorophenyl 3 : 4 : 6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside was unaffected by periodate or iodine indicates that the 2-deoxy-group does not react until after cleavage of an adjacent glycol system.[4] Oxidised aryl 2-deoxyglucosides react with more iodine than the corresponding

[3] Huebner, Lohmar, Ditmer, Moore, and Link, *J. Biol. Chem.*, 1945, **159**, 503.
[4] Cf. Hough and Perry, *Chem. and Ind.*, 1956, 768.

alkyl derivatives and this is consistent with substitution of the aromatic nucleus in addition to the above-mentioned iodination. A feature of the reactions was the production of iodoform when solutions of the oxidation products remained in contact with iodine.

It is clear that care must be exercised in the periodate oxidation of deoxy-sugars, and especially in the choice of method of determination. Other workers [4,5] have drawn attention to the possibility of errors in periodate oxidations where intermediates analogous to those described above are likely to arise, and in such cases Schwarz [5] advises that thiosulphate should be used to titrate the iodine in acid solution. In some instances we have found that a back-titration in which excess of arsenite is added to the oxidised solution before potassium iodide has proved satisfactory. In addition, Aspinall and Ferrier's spectrophotometric method [6] can be used successfully with deoxy-sugars.[7]

Experimental

Periodate Oxidation of 1-Glycosylbenzimidazoles.—(a) *1-β-D-Galactosylbenzimidazole.* The compound (0·0993 g.) in water (90 c.c.) was diluted with 0·25N-sodium periodate (10 c.c.). At noted intervals portions (10 c.c.) were neutralised (1 g. of sodium hydrogen carbonate in 30 c.c. of water), and 10% potassium iodide (1 c.c.) was added. The liberated iodine was estimated after 3 min. by titration with 0·05055N-sodium arsenite with a starch–glycollate indicator. (Control solutions were similarly treated.) Results were:

Time (min.)	10	20	30	45	67	95	130	180	240
$NaIO_4$ uptake (mol.)	0·71	1·07	1·33	1·56	1·86	1·88	1·96	2·01	2·04

After 240 min., titration with 0·0104N-sodium hydroxide to the Methyl Red end-point showed that 0·94 mol. of formic acid was produced.

(b) *1-(2-Deoxy-D-galactosyl)benzimidazole* (II). By a similar procedure this compound (0·1714 g.) gave an apparent periodate consumption of 1·33 mol. after 10 minutes' oxidation. The apparent consumption of periodate is critically dependent on the time for which the products of periodate oxidation are allowed to be in contact with the iodine liberated on destruction of the excess of periodate. Portions (10 c.c.) were removed after 4 hours' oxidation and were treated as follows: (i) titrated immediately with sodium arsenite after addition of potassium iodide; (ii) titrated 10 min.; and (iii) titrated 20 min. after addition of potassium iodide. Results were: (i) 1·86, (ii) 0·59, and (iii) 0·00 c.c. The iodine colour had disappeared completely in sample (iii) before titration. The glycosyl derivative (0·264 g.) in water (20 c.c.) was added to aqueous sodium periodate (0·428 g. in 5 c.c.). After 1 hr. the solution had become turbid and after 5 hr. colourless crystals (5 mg.) were collected, washed with ice-water, and dried (m. p. 90°). They restored the colour to Schiff's reagent and reduced warm ammoniacal silver nitrate. To 1-(2-deoxy-D-galactosyl)benzimidazole (II) (0·264 g.) in water (25 c.c.) was added sodium periodate (0·428 g.), and the solution was halved. To one portion picric acid (0·115 g.) in water (10 c.c.) was added. After 0·5 hr. the *picrate* was collected, washed with water and dried; it had m. p. 103—104°, $[\alpha]_D^{20}$ +31·2° (*c* 2·18 in C_5H_5N) (Found: N, 14·1. $C_{13}H_{14}O_4N_2,C_6H_3O_7N_3$ requires N, 14·25%).

To the other portion of the oxidation mixture, potassium iodide (1 g.) and sodium hydrogen carbonate (1 g.) in water (10 c.c.) were added. Iodine was liberated. The brown colour immediately began to fade, with formation of an amorphous precipitate. After 2 hr. a cream solid (31 mg.) was collected, washed free from iodide ions with water, and dried. This *product* had m. p. 112° (decomp.), $[\alpha]_D^{21}$ +129·5° (*c* 1·56 in C_5H_5N) (Found: C, 39·7; H, 3·7; I, 32·45. $C_{13}H_{13}O_4N_2I$ requires C, 40·2; H, 3·4; I, 32·7%). Solid iodine (1 mol.) was dissolved in the mother-liquors and the brown colour was again discharged, indicating further iodination of the material in solution.

1-2′-Deoxy-D-galactosylbenzimidazole (II) (0·0521 g.) in water (50 c.c.) was oxidised with

[5] Schwarz, *Chem. and Ind.*, 1954, 1000.
[6] Aspinall and Ferrier, *ibid.*, 1957, 1216.
[7] Ferrier and Overend, unpublished results.

0·25N-sodium periodate (10 c.c.). After 1 hr. the solution was neutralised by sodium hydrogen carbonate, and 10% potassium iodide (10 c.c.) and sufficient water to give 100 c.c. were added. At noted intervals portions (10 c.c.) were withdrawn, diluted with water (20 c.c.), and titrated with 0·05055N-sodium arsenite (starch–glycollate indicator) with the following results:

Time (min.)	10	35	60	120	180
I_2 consumed by (III) *	1·67	2·14	2·36	2·54	2·63

* Calc. by assuming 1 mol. of $NaIO_4$ to be consumed in the production of (III).

Periodate Oxidation of 2-(*Polyhydroxyalkyl*)*benzimidazoles.*—(*a*) 2-(D-lyxo-2 : 3 : 4 : 5-*Tetrahydroxypentyl*)*benzimidazole.* The derivative (0·052 g.) in aqueous ethanol (1 : 1, 60 c.c.) was mixed with 0·3N-sodium periodate (10 c.c.) and water (30 c.c.). The uptake of oxidant was determined as before with sodium arsenite, the titration being commenced exactly 3 min. after the liberation of iodine:

Time (min.)	5	15	30	60	120
$NaIO_4$ uptake (mol.)	4·99	4·96	5·06	5·00	4·93

During the oxidation 1·72 mol. of formic acid were liberated.

(*b*) 2-(D-threo-2 : 3 : 4-*Trihydroxybutyl*)*benzimidazole*:

Time (min.)	5	15	30	60	120
NaIO uptake (mol.)	4·16	4·06	3·96	3·94	3·96

1·0 mol. of formic acid was produced.

(*c*) 2-(L-erythro-2 : 3 : 4-*Trihydroxybutyl*)*benzimidazole*:

Time (min.)	5	15	60	90
$NaIO_4$ uptake (mol.)	4·16	4·14	4·10	3·97

Oxidation of Simple Deoxy-sugar Derivatives.—Solutions ($0·5 \times 10^{-2}$M) of the compounds listed in the Table (except for the aryl glycosides) were prepared by dissolving the material in sodium periodate (0·05M; 25 c.c.) and diluting the whole to 50 c.c. with water. (For the aryl glycosides the dilution was with acetone-free methanol.) The procedure for estimation was as described above and the oxidation time was 2 hr. For the first few determinations the iodine was titrated immediately after liberation, but in subsequent assays titration was postponed for periods varying from a few minutes to several hours, and in such cases the flasks were stoppered during storage. All the oxidised solutions which remained in contact with iodine developed a distinct odour of iodoform, and from the solutions of oxidised aryl glycosides iodoform separated and was collected and identified.

The authors thank Professor M. Stacey, F.R.S., for his interest and encouragement. They are indebted to the Department of Scientific and Industrial Research and the Colonial Products Research Council for Maintenance Grants (to A. J. C. and E. J. H. respectively).

CHEMISTRY DEPARTMENT, UNIVERSITY OF BIRMINGHAM.
CHEMISTRY DEPARTMENT, BIRKBECK COLLEGE,
UNIVERSITY OF LONDON.

[*Received, October 23rd,* 1958.]