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The Chlorine Oxidation of Glycosides

I. Oxidation of Methyl β-Glucoside

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The use of chlorine as a bleaching agent is of great technical importance. In the cellulose industry it is employed to remove non-cellulosic material from wood pulp and to destroy colouring matter which is retained in the fibres. This process, however, combined with subsequent alkali treatment of the pulp, involves considerable losses of cellulose [1]; the precise manner in which the cellulose is thus degraded is at present unknown, and an investigation of the reactions concerned is therefore of obvious interest.

Existing knowledge of the effect of halogens on carbohydrates is chiefly confined to the monosaccharides [2]. The reaction between free sugars and halogens has been extensively studied under various conditions, and the conversion of glycosides into uronides by the action of hypobromite has been observed. The action of free chlorine or bromine on simple glycosides has, however, apparently not been studied (reactions occurring in the aglycone are disregarded), and little is known of their action on polysaccharides in general.

In considering the problem of the degradation of cellulose by chlorine, it is an open question whether the attack of the halogen occurs at especially weak points in the cellulose molecule such as have been suggested to exist, or merely at statistically distributed centres throughout a whole chain of equivalent glucose units. However it seemed that in any case the most satisfactory approach to the problem would be to study first the action of chlorine water on some simple glycosides, as model substances from which crystalline reaction products might be expected. The first of these investigations has been carried out with methyl β-glucoside.

An aqueous solution of methyl β-glucoside was subjected to a slow continuous stream of chlorine gas, passing at a rate sufficient to keep the solution

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saturated. At regular intervals the optical rotation of the solution was determined, and the values obtained clearly showed that a reaction was taking place. The initially negative rotation steadily increased, approximately following a first order reaction, and reached a maximum after about 14 days; after this the rotation slowly decreased. During the reaction hydrochloric acid was formed, and also organic acids as was indicated by the fact that the optical rotation of the solution changed considerably on neutralisation. The amounts of these acids present were periodically determined; the total concentration of acids, estimated by neutralisation, was always greater than the concentration of hydrochloric acid, determined by Mohr titration. Portions of the solution were also treated at intervals with periodic acid; the consumption of reagent, initially 1.9 moles per mole of methyl β-glucoside, steadily increased and reached a maximum value of 3.7 at about the same time as maximum optical rotation was observed, after which it decreased. This high periodic acid consumption pointed to the presence of such possible oxidation products as gluconic and glucuronic acids, both of which would theoretically consume 4 moles of the reagent. Gluconic acid was actually isolated from the reaction mixture (after 14 days' chlorination) as the calcium salt and was characterised as the phenylhydrazide; from the yield of these derivatives it was concluded that at least 50 % of the methyl glucoside had been converted to gluconic acid during the oxidation. The identity of the product was confirmed by oxidation of the calcium salt by Ruff's method to give D-arabinose. However as both the optical rotation and the periodic acid consumption were found to pass through maximum values, it was clear that gluconic acid could not be the only product of the reaction, and it was decided to attempt analysis of the mixture by paper partition chromatography.

The chromatographic experiments were carried out with a butanol-acetic acid-water mixture as solvent. A solution of methyl glucoside, which had been chlorinated until it just showed the maximum values referred to above, was treated to remove hydrochloric acid. It then gave a chromatogram which, on development with ammoniacal silver nitrate, exhibited five distinct spots. Two of these were identical with the spots given by a solution of D-gluconic acid chromatographed under the same conditions. Alternative development of these chromatograms with methyl orange caused only the upper spot in each case to appear as a red colouration, the lower spot being absent; the former thus corresponds to gluconic acid itself and the latter to a lactone. The equilibrium between the acid and the lactone in solution is established sufficiently slowly to render chromatographic separation possible, but nevertheless when silver nitrate was used as developing reagent there was always a faint colour between the two spots. Two other spots on the chromatogram of the reaction

mixture were found to be given also by 5-ketogluconic acid * under the same conditions, one again doubtless corresponding to the free acid and the other to a lactone. These spots, from both the unknown and the authentic sources, could be alternatively developed by resorcinol in alcoholic hydrogen chloride, a specific reagent for ketoses.

Similar experiments were carried out with a solution of methyl β-glucoside which had been chlorinated for a period of 40 days. The chromatograms obtained showed that this also contained both gluconic acid and 5-ketogluconic acid but suggested that the proportion of keto-acid present was now rather higher than in the solution oxidised for 14 days only. It therefore appeared probable that the primary product of the oxidation of methyl β-glucoside is gluconic acid and that the latter undergoes further oxidation to give the keto-acid. This conclusion was supported by the results of an additional experiment in which gluconic acid itself was oxidised with chlorine for 14 days under the same conditions as the methyl glucoside. The resulting solution gave a chromatogram which when developed with ammoniacal silver nitrate was almost identical with the chromatogram of oxidised methyl glucoside, showing all five spots found on the latter including the two corresponding to 5-keto-gluconic acid and its lactone. It is of interest to note that Hart and Everett [3] have shown that in the prolonged oxidation of glucose by bromine water, the gluconic acid first formed can be further oxidised to 5-ketogluconic acid. The formation of the keto-acid was finally confirmed by its isolation as a barium salt from the solution of methyl glucoside oxidised for 40 days, and subsequent conversion to the brucine salt which had physical properties in agreement with those recorded in the literature [3].

The compound corresponding to the fifth spot which appeared on the chromatograms of both oxidised methyl glucoside and oxidised gluconic acid has not been identified, but it is certainly only a minor product of the reaction.

The formation of gluconic acid by the action of chlorine upon methyl β-glucoside is an unexpected result. The reaction cannot proceed by way of initial hydrolysis of the glycoside group and subsequent oxidation since it has been shown that at room temperature and in the presence of hydrochloric acid of the maximum concentration produced during the oxidation (i. e., about 2N), methyl β-glucoside is quite stable. The actual mechanism of the oxidation is at present obscure, but it is possible that it proceeds by way of a chloro-derivative of the basic structure shown in the inset, which is formed slowly but undergoes rapid hydrolysis to give ultimately gluconic acid. In this way

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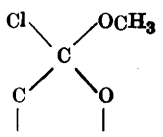

the formation of the latter would approximate to a first order reaction as is found in practice. Some analogous substances to this hypothetical intermediate are described in the literature, for example bromoformaldehyde dimethyl acetal, $BrCH(OCH_3)_2$[4].

The action of chlorine on methyl α-glucoside has also been investigated under similar conditions to those employed with the β-isomer. The reaction was very much slower than with the latter, and after 32 days 66 % of the glucoside could be recovered unchanged. Chromatography of the solution, however, indicated the presence of small amounts of gluconic acid. This difference in reactivity is not unexpected, since it has been shown that while α- and β-glucose both yield gluconic acid by the action of bromine water, the reaction with the β-isomer is 35 times faster than with the α-sugar [5].

On the basis of these results it is possible to formulate a tentative explanation of the degradation of cellulose by chlorine, but further studies which are now in progress on the oxidation of other glycosides, *e. g.* cellobiosides, should render more definite conclusions possible.

EXPERIMENTAL

Action of chlorine on methyl β-glucoside

A slow stream of chlorine gas was passed through a 0.5 M solution of methyl β-glucoside at room temperature with the exclusion of direct light. At appropriate time intervals small portions of the solution were withdrawn, the excess of chlorine removed by aeration for 10—15 minutes, and the following determinations carried out:

a. Determination of optical rotation (2 dm tube).

b. Estimation of total acid present, by titration of the solution (1 ml) with 0.1 *N* sodium hydroxide solution, with gentle warming and using phenolphthalein as indicator, until a permanent pink colour resulted. (Before the final end-point was reached in each titration, vanishing end-points were obtained.)

c. Estimation of hydrochloric acid present, by neutralisation of the solution (1 ml) with sodium bicarbonate and then titration with 0.1 *N* silver nitrate solution by Mohr's method.

d. Determination of periodic acid value. The solution (1 ml) was treated with an excess of a 0.1 *M* solution of periodic acid for 24 hours. After neutralisation with sodium bicarbonate the excess of periodic acid was determined by the arsenite-iodine method. The results of these determinations are shown in Table 1, and in addition the amount of acids present other than hydrochloric acid, calculated by difference from values (b) and (c).

At longer time intervals larger portions of the solution were removed for qualitative examination. After the chlorination had proceeded for 14 days, one such portion was withdrawn and the excess of chlorine removed by aeration. The solution (A) was then found to have a moderately strong reducing power towards Fehling's solution, but tests

for the presence of carbonyl compounds with the usual reagents, *e. g.* 2,4-dinitrophenylhydrazine, gave negative or inconclusive results.

Preparation of calcium salts

The solution (A) (10 ml) was neutralised with silver carbonate, filtered and then saturated with hydrogen sulphide, excess of which was removed by aeration after filtering off silver sulphide. The resulting solution (a small portion of which was reserved for chromatographic examination, see below) was neutralised with calcium carbonate whilst warmed on the water bath, and then filtered; the filtrate was concentrated under reduced pressure to a volume of 5 ml, and the residue poured with stirring into alcohol (100 ml). The calcium salts (0.87 g) thus precipitated were amorphous and attempts to purify them by crystallisation were unsuccessful. The product showed $[\alpha]_{20}^{D}$ + 7.5° in water, c = 1; the value recorded for calcium gluconate is + 8.5°. It was found to contain no methoxyl group, which demonstrated that methyl glucuronide was not present in the reaction mixture.

Table 1. Chlorination of methyl β-glucoside.

Reaction time, days	α_D	Conc. of HCl, equiv./l	Conc. of other acids, equiv./l	HIO_4 consumption, mol./mol.
0	− 6.26°	0	0	1.91
1	− 3.96	0.38	0.12	2.50
2	− 2.55	0.58	0.20	2.96
4	− 0.84	0.83	0.26	3.43
6	+ 0.33	1.04	0.37	3.62
10	+ 1.37	1.35	0.43	3.68
14	+ 1.57	1.62	0.53	—
19	+ 1.50	1.86	0.58	3.58
25	+ 1.43	2.25	0.72	3.34

Isolation of gluconic acid as the phenylhydrazide

Another portion (10 ml) of the solution (A) was freed from hydrochloric acid as described above and the final filtrate concentrated to a volume of about 10 ml. Glacial acetic acid (1.5 ml) and phenylhydrazine (1 ml) were added, the mixture heated on the water bath for 1 hour and then filtered while still hot to remove amorphous material. The solution was allowed to stand overnight at 0° and the yellow crystals which had separated were then collected and washed with ether. The product (0.66 g) was dissolved in boiling alcohol (350 ml), filtered while hot, and the filtrate kept at 0° for 3 hours. Colourless crystals (0.39 g, 27 % of the theoretical yield) were then obtained, which after two further recrystallisations from alcohol had m. p. 196° * (decomp.), undepressed on

* All melting points uncorrected.

admixture with authentic gluconic acid phenylhydrazide, and $[\alpha]_D^{20}$ + 13° in water, c = 1 (the value given in the literature is $[\alpha]_D$ + 12° in water, c = 2).

$C_{12}H_{18}O_6N_2$ (286.3)	Calc.	N 9.79
	Found	» 9.89

Preparation of D-arabinose from the calcium salts

The calcium salts, prepared as described above, (2 g) were oxidised with hydrogen peroxide in the presence of ferric sulphate according to the method of Hockett and Hudson [6]. This gave D-arabinose (0.1 g) which after recrystallisation from absolute methanol had m. p. 152—154°, undepressed on admixture with authentic material. The low yield of arabinose obtained was probably due to the small scale on which the reaction was carried out; under the same conditions authentic calcium gluconate (2 g) gave D-arabinose (0.2 g).

Isolation of 5-ketogluconic acid as the brucine salt

After the chlorination of methyl β-glucoside had proceeded for 40 days, a portion (50 ml) of the solution was withdrawn and the hydrochloric acid present removed as described above. It was then treated with an excess of barium carbonate at 40—45° for 2 hours, filtered, and the filtrate concentrated under reduced pressure to a volume of 5 ml. Alcohol (40 ml) was added, and after a few hours the precipitated barium salts were collected, washed with alcohol and ether and dried. Yield, 1.70 g. The barium salts (1.0 g) were dissolved in water (2—3 ml) and the barium removed quantitatively by addition of N sulphuric acid. After filtration, alcohol (12 ml) was added and then a 10 % alcoholic brucine solution (38 ml). After allowing the mixture to stand at 0° overnight, the crystalline brucine salt was collected, washed with alcohol and ether. Yield, 0.8 g. Recrystallisation from water gave brucine 5-ketogluconate as needles, m. p. 172—173°, $[\alpha]_D^{20}$ —26° ± 2° (micro-determination). Hart and Everett [3] give m.p. 174—175°, $[\alpha]_D$ —24°.

Action of hydrochloric acid on methyl β-glucoside

A 0.5 N solution of methyl β-glucoside in 2 N hydrochloric acid was kept at room temperature and the optical rotation periodically determined. After 23 days the α_D value had only changed from —6.36° to —6.24° (2 dm tube). The solution was then freed from hydrochloric acid in the usual way, and after evaporation to dryness under reduced pressure and recrystallisation of the residue from alcohol a 95 % recovery was obtained of methyl β-glucoside, identical with the starting material.

Action of chlorine on D-gluconic acid

A 5 % aqueous solution of D-gluconic acid (prepared from the δ-lactone) was treated with chlorine gas under the same conditions as methyl β-glucoside. After the reaction had proceeded for 14 days, the hydrochloric acid present was removed as in previous experiments, and the solution obtained subjected to chromatographic examination (see below).

Action of chlorine on methyl α-glucoside

This experiment was carried out exactly as described earlier for the β-glucoside, using the same concentration of substance. After the passage of chlorine had been continued for 32 days, the optical rotation of the solution had only changed slightly, from the initial α^D value of + 30.82° to + 29.26° (2 dm tube). The periodic acid consumption showed no change within the limits of experimental error. The solution was then freed from hydrochloric acid, and a portion examined chromatographically (see below). The remainder was evaporated to dryness under reduced pressure and the residue recrystallised from alcohol to give a 66 % recovery of unchanged methyl α-glucoside.

Chromatographic examination of the reaction mixtures

The chromatographic experiments were carried out on Whatman No. 1 paper, using as solvent a mixture of butanol (40 %), acetic acid (10 %) and water (50 %). The solutions from the oxidation reactions, after removal of hydrochloric acid as described, were adjusted to concentrations of 4—5 % with respect to the carbohydrate taken; the solutions of reference compounds used were all approximately 2 %. One drop of the appropriate solution was used for each chromatogram. After application of the drops, the papers were normally allowed to remain in the vapour of the aqueous phase for one hour before the apparatus was charged with the other phase, and the chromatograms were then run for an average time of 16—18 hours. The papers were dried at 100°, then developed. The following developing reagents were employed: ammoniacal silver nitrate (5 %), resorcinol (3 %) in alcoholic hydrogen chloride (5 %), and methyl orange (ordinary indicator solution). In the case of the first two reagents, the papers were heated at 110° after spraying; in the case of methyl orange the papers were dried for a prolonged period (1 hour) before spraying to remove residual acetic acid. The silver nitrate solution proved very successful as a developing reagent for gluconic acid in spite of the absence of reducing groups in the latter.

Table 2. Chromatographic experiments.

Substance chromatographed	Reagent	I	II	III	IV	V
Chlorinated methyl β-glucoside	$AgNO_3$	+	+	+	+	+
	Resorcinol	—	—	+	+	—
	Methyl orange	—	+	+	—	—
Chlorinated gluconic acid	$AgNO_3$	+	+	+	+	+
Chlorinated methyl α-glucoside	$AgNO_3$	—	(+)	—	—	(+)
Gluconic acid (authentic)	$AgNO_3$	—	+	—	—	+
5-Ketogluconic acid (authentic)	$AgNO_3$	—	—	+	+	—
	Resorcinol	—	—	+	+	—

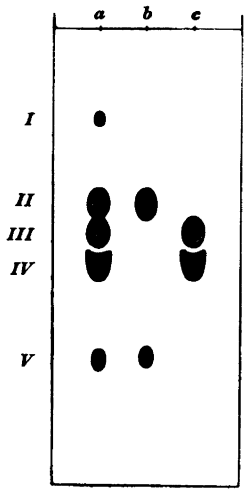

Fig. 1. Typical chromatograms.

a = Chlorinated methyl β-glucoside
b = Authentic gluconic acid
c = » 5-ketogluconic acid

The results of the chromatographic experiments are summarised in Table 2. In addition to the reference compounds mentioned in the table, glucuronic acid, saccharic acid, glucose and arabinose were also chromatographed simultaneously with the methyl β-glucoside mixture, but no indications of the presence of these substances in the latter were obtained. (The chromatograms were developed with ammoniacal silver nitrate in the case of the two acids, and with aniline hydrogen phthalate reagent in the case of the aldoses.)

Explanation of table. The Roman numerals refer to the five spots which appear on the chromatogram of oxidised methyl β-glucoside, as shown in the accompanying diagram (Fig. 1). Presence or absence of these spots on the other chromatograms is indicated by a positive or negative sign respectively; the two positive signs in brackets demote weak colourations.

SUMMARY

The action of chlorine water on methyl β-glucoside has been studied and it has been shown that the chief product is D-gluconic acid, which is slowly oxidised further to 5-ketogluconic acid.

Methyl α-glucoside also seems to undergo a similar reaction but at a very much slower rate.

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