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The Chlorine Oxidation of Glycosides

III.* Oxidation of Methyl β-Cellobioside

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Parts I and II of this series have dealt with the oxidation of simple methyl glycosides with chlorine water. It was found that the β-glycosides were oxidized to glyconic acids under conditions which precluded initial hydrolysis of the glycoside. The intention of this work is to study the degradation of cellulose by bleaching with chlorine, the methyl β-glycosides being regarded as simple model substances. In this communication similar experiments with more cellulose-like model substances, methyl β-cellobioside and cellobionic acid, are reported.

The reaction of methyl β-cellobioside with chlorine water was carried out and followed by analytical determinations as for the corresponding reaction of methyl β-glucoside [1]. Calculations from optical rotation measurements indicated that two reactions of different velocities took place. The faster reaction occurred at about the same rate as the reaction of methyl β-glucoside with chlorine water, while the second appeared to be two or three times slower than this and predominated during the second week of chlorination.

At intervals portions of the reaction mixture were withdrawn, freed from chlorine and hydrochloric acid, and analyzed in paper partition chromatograms. A solvent mixture of butanol, ethanol, and water was used. The spots were in most cases detected with a silver nitrate reagent according to the method of Trevelyan, Procter and Harrison [2]. This reagent proved very useful and gave dark colourations with all the substances encountered in this work.

The acids and lactones which were present gave chromatograms which were difficult to interpret on account of poor separation of the spots. It was

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therefore decided to convert the acids into derivatives which could be more readily separated, and the phenylhydrazides were found to be convenient for this purpose. Norris and Campbell [3] have used phenylhydrazine hydrochloride to convert keto acids into their phenylhydrazones before chromatographic identification of gluconic acid. In the present investigation samples of the reaction mixture were heated with phenylhydrazine and non-reducing acids thus partly converted into phenylhydrazides. These derivatives travelled much faster than the free acids on the chromatogram, and they gave strong spots, easy to identify. Reducing acids appeared only faintly or were carried into a dark zone near the solvent front.

Examination of the solutions by this technique showed that cellobionic acid is readily formed in the early stages of the chlorination reaction, while gluconic acid is produced more slowly and accumulates during the first two weeks. Traces of methyl β-glucoside appeared transiently. A separate chlorination experiment showed that cellobionic acid itself is oxidized to gluconic acid.

These results indicate that the methyl-glucoside bond in methyl β-cellobioside and the disaccharide linkage in cellobiose derivatives in general are cleaved in the same manner as in methyl β-glycosides, although the latter type of linkage undergoes cleavage less readily than the former.

Gluconic acid is not a stable end product in the reaction with chlorine water; it is slowly oxidized further to 5-ketogluconic acid [1]. Saccharic acid was also detected in chromatograms of chlorinated gluconic acid. Saccharic acid is obviously responsible for the "fifth spot" met with in the previous work [1], though this fact was not demonstrated at the time.

The solutions of chlorinated cellobionic acid and methyl β-cellobioside contain unidentified components, which are not present in solutions of chlorinated gluconic acid or methyl β-glucoside. The positions of these components on the chromatograms suggest that they are derivatives of cellobionic acid. Two of these spots could be developed with a spraying reagent for keto-compounds and thus probably arise from ketocellobionic acids.

Attempts to prepare cellobionic acid phenylhydrazide in a crystalline state failed, although its preparation has been reported [4]. Gluconic acid was isolated in a small yield as the phenylhydrazide from the solution of chlorinated methyl β-cellobioside. Saccharic acid was isolated from a solution of chlorinated gluconic acid as the potassium hydrogen salt in a yield of 6 % and identified as the dibenzimidazole derivative.

Methyl β-cellobioside and cellobionic acid were treated with 2 *N* hydrochloric acid, this corresponding to more than the maximum hydrochloric acid strength produced in a 13-day chlorination. Chromatographic examina-

tion of the solutions showed that it took 10 days before detectable amounts of hydrolysis products appeared. The reaction with chlorine, therefore, cannot take place with an initial hydrolysis of the glucosidic bonds.

EXPERIMENTAL

Action of chlorine on methyl β-cellobioside and cellobionic acid. — A 0.25 *M* solution of methyl β-cellobioside was treated with chlorine and determinations carried out following the earlier procedure [1]. The results are given in Table 1. Samples withdrawn were at once freed from chlorine by aeration and from hydrochloric acid by successive treatment with silver carbonate and hydrogen sulfide.

Table 1. Chlorination of methyl β-cellobioside.

Reaction time		α_D	Conc. of HCl.
days	hours		equiv./l
0	0	− 3.35	0
0	20	− 2.78	
1	20	− 2.28	
2	20	− 1.82	
4	0	− 1.48	
5	20	− 1.08	0.77
9	0	− 0.53	1.08
12	22	− 0.16	
15	21	+ 0.06	1.97
23	0	+ 0.41	

Cellobionic acid (0.25 *M*) was chlorinated in the same way. The α_D-values changed only slightly.

Chromatographic examinations. — The chromatograms were run on Whatman No. 1 paper and developed for 22 hours by the descending technique with a mixture of butanol (40 %), ethanol (10 %), and water (50 %). The spots were detected with a silver nitrate reagent [2] as already mentioned. When reducing acids were studied it was found more convenient to omit the phenylhydrazine treatment, and to develop the spots directly with resorcinol reagent.

Solutions to be treated with phenylhydrazine were heated (1 ml taken) in small, sealed Pyrex tubes with phenylhydrazine (0.1 ml) for one hour on the steam bath. The tubes were turned a couple of times during heating in order to mix the contents. After cooling the mixture was allowed to settle, and the clear solution was applied to the paper.

R_F-values for the pure compounds and for the phenylhydrazides are given in Table 2. The results of the chromatographic experiments are summarized in Table 3.

Table 2. R_F-values.

Compound	Compound	Phenylhydrazide
Methyl β-cellobioside	0.14	
Methyl β-glucoside	0.30	
Cellobionic acid	0.02	0.38
Gluconic acid	0.04	0.57
5-Ketogluconic acid	0.05	0.45—0.60 a)
Saccharic acid	0.02	0.22; 0.85 b)

a) Probably the phenylhydrazone. The spot trailed and was difficult to observe.
b) Most probably the mono- and the diphenylhydrazides respectively.

Table 3. Chromatographic examinations.

Chlorinated solution	Time of chlorination, days	Compound					
		Methyl β-cellobioside	Methyl-β-glucoside	Cellobionic acid	Gluconic acid	5-Ketogluconic acid	Saccharic acid
Methyl β-cellobioside	2	+ +	−	+ +	(+)		
	5	+	(+)	+ +	+		
	9	(+)	(+)	+ +	+ +	+	+
	13	−	−	+	+ +	+	+
Cellobionic acid	9			+	+ +	+	+
	27			(+)	+ +	+	+
Gluconic acid	27				+ +	+	+

Isolation of gluconic acid as the phenylhydrazide. — Methyl β-cellobioside solution, chlorinated for 9 days, (5 ml) was freed from chlorine and hydrochloric acid and evaporated to 0.5 ml. Phenylhydrazine (0.25 ml) was added and the mixture heated 1.5 hours on the steam bath. The excess of phenylhydrazine was extracted with ether (5 × 10 ml) and a brown precipitate (43 mg) collected. One recrystallization from alcohol gave a slightly coloured product (28 mg) with m.p. 187° (decomp.) *. The yield was 7 % calculated on the methyl β-cellobioside taken. Further recrystallizations yielded pure gluconic acid phenylhydrazide, m.p. 195° (decomp.).

Isolation of saccharic acid as the dibenzimidazole. — Gluconic acid (0.5 *M*) in hydrochloric acid (3 *N*) was chlorinated for 27 days. The solution (40 ml) was freed from chlorine

* All melting points uncorrected.

and hydrochloric acid. Potassium hydroxide solution was added until the solution became alkaline, and the mixture was then heated for 45 min. on the steam bath. After cooling acetic acid was added to pH 3.5 [5]. The solution was concentrated to 10 ml and acetic acid (1 ml) was added. After standing for 3 days at 0° the precipitate was collected and washed with 30 % ethanol. 0.53 g of an almost colourless compound was obtained. One recrystallization from hot water gave potassium hydrogen saccharate (0.31 g) which decomposed at 184°. Yield 6.3 % calculated on the gluconic acid taken.

The potassium hydrogen saccharate (0.31 g) was condensed with *o*-phenylene diamine following the procedure of Lohmar, Dimler, Moore, and Link [6]. Saccharic acid dibenzimidazole (0.27 g), identical with an authentic preparation, was obtained. It had m.p. 230° (decomp.) and $[\alpha]_D^{20}$ + 61° (c = 2 in 5 % citric acid; microtube). Lohmar *et al.*[6] reported m.p. 238 (d.) and $[\alpha]_D^{25}$ + 60.3°.

Action of hydrochloric acid on methyl β-cellobioside and cellobionic acid. — A solution of methyl β-cellobioside (c = 1.72) in hydrochloric acid (2 N) was kept at room temperature. The a_D-value changed from — 0.54 to — 0.51 in 16 days (2 dm tube). Paper chromatograms were prepared, and after 10 days a faint spot of glucose became visible.

Cellobionic acid (c = 1.79) in hydrochloric acid (2.1 N) was treated similarly and with the same result.

SUMMARY

Chlorination experiments with methyl β-cellobioside in aqueous solution have indicated that both glucosidic bonds are cleaved in the same manner as has previously been demonstrated for methyl β-glycosides. The methyl-glucoside bond suffers cleavage more rapidly than the bond between the two glucose units. Cellobionic acid is formed as an intermediate which is further oxidized to gluconic acid. Saccharic acid has been isolated after prolonged chlorination of a gluconic acid solution.

A technique for the chromatographic identification of non-reducing carbohydrate acids as their phenylhydrazides has been developed.

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