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Alkaline Hydrolysis of Glycosidic Linkages

II.* Investigation of Cellobitol, Lactitol and Maltitol

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The hydrolysis of cellobitol, lactitol and maltitol in alkaline, aqueous solutions at 170° has been studied as part of an investigation of the decomposition of carbohydrates during alkaline pulping. All substances were hydrolysed at roughly comparable rates. D-Glucitol, 1,4-anhydro-D-glucitol and levoglucosan were formed from cellobitol, the first two together with 1,6-anhydro-D-galactose were formed from lactitol. Another, isomeric glucitol β-glucoside was formed from cellobitol and an analogous β-galactoside from lactitol.

It was briefly reported in Part I * that some glycosides, such as methyl β-glucoside and cellobitol, although generally considered to be stable to alkali, were decomposed under the conditions of sulphate or soda cooking, *e. g.* in alkaline solution at 170°. In the present paper a more detailed investigation of some glucitol glycosides is reported.

Cellobitol, lactitol and maltitol were prepared from the corresponding disaccharides by reduction with sodium borohydride [1]. Cellobitol was obtained in a crystalline state, anhydrous, m. p. 149—150°, $[\alpha]_D^{20}$ + 8°, and with one mole of water of crystallisation, m. p. 107—108°. The previously recorded m. p. is 133° [2], which might indicate that the sample investigated consisted of a mixture of the two forms above. Lactitol and maltitol were obtained as syrups.

The glycosides were dissolved in 10 % aqueous sodium hydroxide and heated in stainless steel autoclaves at 170° for various times. The alkali, together with the acids formed during the reaction, was removed by ion exchange and the yield of non-ionic material, a mixture of starting material and the products of reaction, was determined. The yields are given in Table 1. If the glucitol glycosides were completely degraded into glucitol and acidic products, the final yield should be 53 %.

* Part I, *Svensk Papperstidn.* **59** (1956) 531.

Table 1. Yield of non-ionic material, in percentage of starting material, after treatment of cellobitol, lactitol and maltitol with 10 % sodium hydroxide at 170°.

Time, h	Cellobitol, yield	Lactitol, yield	Maltitol, yield
5	73	86, 92	85, 93
24	65	61	60
48	49	53	45

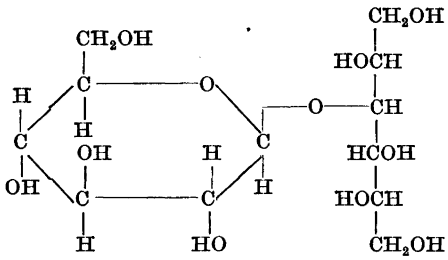
Due to mechanical losses, difficulties in drying the amorphous starting materials and products and to imperfect temperature control, the values in Table 1 are not very accurate, but they show that all the substances have been degraded to a considerable extent and at rates that are of the same order of magnitude.

A chromatographic investigation of the products from the cellobitol (I) revealed the presence of unchanged starting material, a substance with the same R_F-value as glucitol and of two substances with higher R_F-values. The product was fractionated on a cellulose column and the pure components were isolated. The substance with the highest R_F-value was identified as levoglucosan (II), the next as 1,4-anhydro-D-glucitol (arlitan) (III); both substances were identical with authentic specimens. The D-glucitol and cellobitol were also characterised. A small amount of material with an R_F-value slightly faster than that of cellobitol was also obtained.

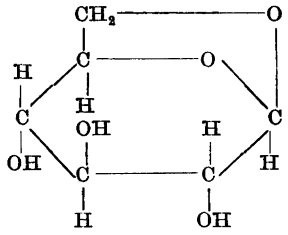
Analogous or identical substances were obtained from lactitol, that is 1,6-anhydro-D-galactose, 1,4-anhydro-D-glucitol, D-glucitol, unknown material and unchanged starting material. The presence of glucitol and 1,4-anhydro-D-glucitol in the products obtained from the maltitol was demonstrated by paper chromatography.

The stability to alkali of the substances formed was investigated. Faint traces of 1,4-anhydro-D-glucitol and another substance with somewhat lower R_F-value, possibly 1,5-anhydro-D-glucitol (polygalitol) could be observed after treatment of D-glucitol with 10 % sodium hydroxide at 170° for 24 h. Some D-glucitol was also formed when 1,4-anhydro-D-glucitol was given an analogous treatment. Levoglucosan and 1,6-anhydro-D-galactose were more labile. The yield of non-ionic material, in this case consisting of pure starting material, decreased rapidly with the time of treatment in alkali. The percentages of unchanged levoglucosan after 1, 3 and 5 h in 10 % sodium hydroxide at 170° were 58, 35 and 25, respectively. The corresponding figures for 1,6-anhydro-D-galactose were 85, 69 and 53 % after 2, 4 and 6 h, respectively. Thus these anhydrides are considerably more labile than the glucitol glycosides and the percentage present in the reaction mixtures should consequently be quite low. As levoglucosan is the most labile of the two, it should be present in the lowest concentration, as was the case.

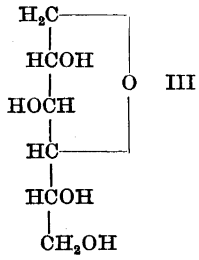
Levoglucosan and 1,6-anhydrogalactose are also formed by the alkaline hydrolysis of phenyl β-glucosides and β-galactosides, respectively. These reactions involve the intermediate formation of a 1,2-anhydrosugar (*cp.* Ref.[3]),



I



II



III

and it is possible that the alkaline hydrolysis of cellobitol and lactitol follows a similar route. As 1,4-anhydro-D-glucitol is formed in a higher yield in these reactions than in the analogous alkaline treatment of D-glucitol, it is evident that this is not the only mechanism by which the glycosidic linkage is cleaved. A reasonable assumption is that the 1,4-anhydro-D-glucitol is formed by a nucleophilic attack on $C_{(4)}$ of the primary hydroxyl at $C_{(1)}$ (as alkoxide) in the glucitol unit. This reaction may be preceded by the formation of an ethylene oxide ring either between $C_{(4)}$ and $C_{(5)}$ or between $C_{(3)}$ and $C_{(4)}$; of these alternatives the former appears to be the more plausible.

Fission between the glycosidic oxygen and $C_{(1)}$ in the glucose unit should therefore yield 1,6-anhydrosugar and glucitol, fission between this oxygen atom and C_4 in the glucitol unit, on the other hand, should yield glucose and 1,4-anhydro-D-glucitol. A direct attack of hydroxyl ion, either on $C_{(1)}$ in the glucose unit or on $C_{(4)}$ in the glucitol unit, should give glucose and glucitol. The glucose, of course, is rapidly decomposed into acidic products under the experimental conditions. No formation of levoglucosan from maltitol was observed, which is in agreement with the mechanism postulated; however, a small amount of this substance could easily have escaped detection.

As mentioned above, a substance moving only slightly faster on the paper chromatogram than the starting material was obtained in a low yield both from cellobitol and lactitol. The substance from cellobitol was obtained chromatographically pure and on hydrolysis yielded glucose and glucitol, identified by paper chromatography in various solvent. The specific rotation was low, $+20°$, indicating a β-glucosidic structure. The substance therefore should be a D-glucitol β-D-glucoside, isomeric with cellobitol. Analytical periodate oxidations did not give further information about its structure, as the substance

was obviously not pure. The substance from the lactitol cook was obtained chromatographically pure and on hydrolysis yielded galactose and glucitol, identified chromatographically. The formation of these substances is of considerable interest and demonstrates the possibility of transglycosidation reactions during alkaline pulping. Transglycosidations during alkaline treatment of phenyl glycosides are known [3] and have recently been further illustrated [4]; they most probably proceed *via* the 1,2-anhydrosugar. The present case seems, however, to be the first observation of a reaction of this type, starting from an alkyl glycoside.

EXPERIMENTAL.

All melting points are corrected. All evaporations were carried out under reduced pressure. Optical rotations were determined in aqueous solutions, $c \simeq 2$.

Chromatography. Whatman No. 1 and 3 MM filter papers and Whatman ashless cellulose powder, standard grade, were used.
Solvent systems: A. Methyl ethyl ketone, saturated with water.
B. Ethyl acetate-acetic acid-water, 3:1:1.
Developers: Silver nitrate-sodium hydroxide and, for the reducing sugars, anisidine hydrogen chloride.

Preparation of the glucitol glycosides. Cellobitol, lactitol and maltitol were prepared from the corresponding disaccharides by reduction with sodium borohydride following the procedure of Abdel-Aker *et al.*[1]. After deionisation, the boric acid was removed by repeated distillations with methanol. Lactitol and maltitol were obtained as syrups. Crystallisation of cellobitol from methanol yielded the anhydrous substance, m. p. 149–150° $[\alpha]_D^{20}$ +8°. By crystallisation from aqueous ethanol it was obtained with water of crystallisation, m. p. 106–108°. After drying in a vacuum at 75° over phosphorus pentoxide, the loss in weight corresponded to 1 mole water per mole cellobitol.

Alkaline treatment, procedure. A known amount of substance was dissolved in 7–10 parts of 10 % aqueous sodium hydroxide and transferred into a stainless steel autoclave. The air was replaced with nitrogen and the autoclave heated at 170° for various times. The heating was performed either in a gas-heated oil bath (shorter reaction times) or in an electrically heated oven. The cooled solution was then filtered through a column, prepared from equal parts of the ion exchange resins Amberlite IR 120 and Dowex 2, in the hydrogen and hydroxyl state, respectively. The eluate and washings were concentrated to dryness and weighed. The yields of non-ionic products which were obtained from cellobitol, lactitol, maltitol, levoglucosan and 1,6-anhydro-D-galactose after alkaline treatment for various times are given in the account above.

Products from the alkaline treatment of cellobitol. Anhydrous cellobitol (8.56 g) was dissolved in 10 % aqueous sodium hydroxide (50 ml), kept at 170° for five hours and worked up as described above. The yield of non-ionic material was 6.24 g. Unchanged starting material (5.1 g) was recovered by crystallisation from 90 % aqueous ethanol. The material from the mother liquors (1.00 g) was fractionated on a cellulose column (20 × 4.5 cm), using solvent system B as eluant. The following fractions, listed in the order in which they eluted from the column, were obtained.

a. Levoglucosan, 33 mg. Recrystallisation from ethanol yielded the pure substance, m. p. 178–180°, undepressed on admixture with authentic material.

b. 1,4-Anhydro-D-glucitol, 475 mg. The substance was further purified by recrystallisation from ethanol. M. p. 116–117°. $[\alpha]_D^{20}$ −22°. The substance was indistinguishable from an authentic sample, prepared according to Soltzberg, Goepp and Freudenberg [5].

c. D-Glucitol, 209 mg. M. p. after recrystallisation from ethanol, 96–97°. The acetate was also prepared, m. p. 101–102°, undepressed on admixture with an authentic sample.

d. Unknown + cellobitol, 76 mg.

e. Cellobitol, 277 mg.

The unknown substance in fraction *d* was further purified by chromatography on thick filter paper, using solvent system B. It had an R_F-value slightly higher than that of cellobitol. The chromatographically pure substance (25 mg) on hydrolysis yielded glucose and glucitol, as identified by paper chromatography. The amorphous product had $[\alpha]_D^{20}$ + 20° and on periodate oxidation with 0.03 N sodium metaperiodate solution rapidly consumed about 7.1 moles of periodate with the formation of 2.7 moles of formic acid. These results would not be expected from a pure hexitol glycoside and indicate that the substance was not pure.

Products from the alkaline treatment of lactitol. Lactitol (2.80 g) was treated with 10 % aqueous sodium hydroxide at 170° for 5 h and worked up as described above. The non-ionic products (2.37 g) were fractionated on a cellulose column, (27 × 4.5 cm), using solvent system A as eluant. The following fractions, listed in the order in which they were eluted from the column, were obtained.

a) 1,6-Anhydro-D-galactose, 0.18 g. The substance was purified by recrystallisation from ethanol. $[\alpha]_D^{20}$ −22°, m. p. 224−226°, undepressed on admixture with an authentic sample.

b) 1,4-Anhydro-D-glucitol (0.18 g). M. p. 116−117°, after recrystallisation from ethanol.

c) Glucitol, 0.40 g.

d) Lactitol, 1.49 g.

The lactitol contained small amounts of a substance with slightly higher R_F-value, which was isolated by chromatography on thick filter paper. It yielded galactose and glucitol on hydrolysis but was not further investigated.

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