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Short Communications

The Oxidation of Glycosides

XIV *. The Oxidation of Methyl β-D-Glucopyranoside with Nitrogen Dioxide

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Nitric acid is an oxidising agent which is known to preferentially oxidise primary alcoholic groups in carbohydrates to carbonyl groups.[1] However, the formation of fragmentation products using this oxidant, as well as the oxidation of aldonic acids to keto-aldonic acids, shows that secondary alcoholic groups are also oxidised to some extent. Oxidation by nitrogen dioxide appears to be more specific. Cellulose can readily be oxidised to a product, "celluronic acid", with a carboxyl content which is more than 20 %.[2] The properties of this product, however, indicate that carbonyl groups are also present. Nabar and Padmanabhan oxidised cellulose with nitrogen dioxide and with a mixture of nitrogen dioxide and oxygen and obtained oxidised celluloses having different properties.[3]

The formation of carbonyl glycosides by the oxidation of methyl β-D-glucopyranoside, chosen as a model for cellulose, has previously been studied using chromium trioxide,[4] chlorine water and aqueous hypochlorite at different pH values [5] and Fenton's reagent.[6] In the present investigation the oxidation of methyl β-D-glucopyranoside with liquid nitrogen dioxide has been studied. The glucoside was dissolved in an excess of the oxidant (the molar ratio oxidant to glucoside was 4:1) and kept at 12° for 5 h. In two similar experiments oxygen and nitrogen, respectively, were passed through the reaction mixture. The neutral reaction products were studied by paper chromatography and electrophoresis and the presence of carbonyl glycosides was indicated. No difference in composition between the products from the two experiments was observed.

In a larger-scale experiment the neutral oxidation products were isolated by a combination of carbon column chromatography, paper chromatography and paper electrophoresis. The four possible monocarbonyl glycosides were obtained in the following yields: methyl β-D-*arabino*-hexopyranosidulose (1.8 %), methyl β-D-*ribo*-hexopyranosid-3-ulose (2.8 %), methyl β-D-*xylo*-hexopyranosid-4-ulose (1.0 %) and methyl β-D-*gluco*-hexodialdo-1,5-pyranoside (0.1 %). The main part of the neutral product was unchanged starting material (22 % was isolated). It is notable that the total amount of carbonyl glycosides is comparable to that obtained by chromium trioxide treatment;[4] both oxidations being carried out under conditions where the total yield of carbonyl glycosides is about a maximum. Chromium trioxide oxidation preferentially gave the 3-keto compound, but with nitrogen dioxide as oxidant the keto glycosides were formed in comparable amounts. The yield of the 4-keto compound is higher than by any of the previously used oxidants. The aldehydo derivative, however, is obviously readily oxidised as would be expected.

The acidic oxidation products, amounting to about 60 % of the reaction mixture, were not studied in detail. It was shown, however, that D-glucaric acid, isolated as its potassium hydrogen salt, was the major acid, indicating that the oxidative demethylation is considerable. This is in agreement with the results of Hardegger and Spitz.[7]

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Experimental. Methyl β-D-glucopyranoside (2.00 g) was dissolved in liquid nitrogen dioxide (1.90 g) in a two-necked flask fitted with an efficient water-cooled condenser carrying a calcium chloride tube. In one experiment a slow stream of oxygen, and in another a slow stream of nitrogen, was passed through the solution, which was maintained at 12° for 5 h. The product was then evaporated under reduced pressure (at 30°), dissolved in water and deionised (Dowex 3 (free base)). Paper chromatographic (solvents: ethyl acetate—acetic acid—water, 3:1:1 and butan-1-ol—ethanol—water, 10:3:5) and electrophoretic (buffer: 0.1 M hydrogen sulphite, pH 4.7 used at 50°) examination of the neutral products from the two experiments revealed similar patterns. Unchanged starting material was the main product together with the four possible mono-carbonyl glycosides and some unidentified products. Methyl β-D-glucopyranoside (10.00 g) in nitrogen dioxide (9.50 g) was kept at 12° for 17 h. The fractionation of the neutral components in the reaction mixture was carried out essentially as previously described (Ref. [4] and earlier papers) by chromatography on a carbon-Celite column and sub-fractionations by chromatography and electrophoresis on thick filter papers. The amounts of carbonyl glycosides isolated are given in the text. They were all shown by paper chromatography and electrophoresis to be indistinguishable from authentic samples. The 3-keto compound, methyl β-D-*ribo*-hexopyranosid-3-ulose, was obtained in the crystalline state, m.p. and mixed m.p. 129—130°.

The acids were recovered from the anion exchange resin as their ammonium salts (6.95 g); a brown, sticky, partially crystalline product. After carbon treatment the acids were converted to their potassium salts, and the relatively insoluble crystalline potassium hydrogen salt (2.37 g) of D-glucaric acid was isolated in the usual way. The product after removal of the cations from the light yellow salt was shown to be indistinguishable from an authentic sample of D-glucaric acid by paper chromatography and electrophoresis (buffer: 0.1 M acetate, pH 4). After one re-precipitation the salt was colourless and its IR-spectrum (KBr) was identical with that of an authentic sample.

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1. Green, J. W. in Pigman, W. *The Carbohydrates*, New York 1957, p. 299.
2. Nevell, T. P. in Whistler, R. L. *Methods in Carbohydrate Chemistry*, New York 1963, vol. III, p. 164.
3. Nabar, G. M. and Padmanabhan, C. V. *Indian Acad. Sci.* Bangalore, *Proc. Section A* **32** (1950) 212.
4. Assarsson, A. and Theander, O. *Acta Chem. Scand.* **12** (1958) 1507.
5. Theander, O. *Svensk Papperstid.* **61** (1958) 581.
6. de Belder, A. N., Lindberg, B. and Theander, O. *Acta Chem. Scand.* **17** (1963) 1012.
7. Hardegger, E. and Spitz, O. *Helv. Chim. Acta* **33** (1950) 337.

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The Use of a Recording Beckman DB Spectrophotometer for the Assay of ATP with a Luciferin-Luciferase Reagent *

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The assay of ATP *** with the luciferin-luciferase reagents is usually carried out with a photofluorometer or a specially designed instrument.[1,2] Manufacturers of the reagents also describe methods with somewhat lower sensitivity utilizing the Beckman DU spectrophotometer. The use of this instrument does not, however, allow registration of the maximum values of the light emission. The procedure therefore

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*** The following abbreviations are used:
ATP, adenosine-5′-triphosphate
GTP, guanosine-5′-triphosphate
ITP, inosine-5′-triphosphate
UTP, uridine-5′-triphosphate.