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Studies on the Synthesis and Isolation of Oxidized Glycosides*

BENGT LINDBERG and K. N. SLESSOR**

Institutionen för Organisk kemi, Stockholms Universitet, Stockholm, Sweden

Oxidation of the anomeric methyl D-xylopyranoside 2,4-phenylboronates with dimethylsulphoxide-acetic anhydride provided the anomeric methyl D-*erythro*-pentopyranosid-3-uloses in high yield. Isolation of these compounds was facilitated by chromatography on strongly basic ion exchange resin in the bisulphite form. The utility of such a separation method enabled the isolation of methyl α-D-*ribo*-hexopyranosid-3-ulose and α-D-*ribo*-hexopyranosyl-3-ulose-β-D-fructofuranoside from the direct oxidation of methyl α-D-glucopyranoside and sucrose, respectively.

The importance of keto sugars in the chemical synthesis [2] and biosynthesis [3] of branch chain sugars has been described. Recently keto sugar nucleotides have been shown to be intermediates in the biosynthesis of different sugars from glucose.[4,5] It was, therefore, of interest to investigate some aspects of the chemical synthesis and isolation of keto sugars.

Syntheses of unsubstituted pento-and hexo-pyranosiduloses have been reported by various authors using two main methods. Unspecific oxidation of glycosides with oxidants like chromic acid in pyridine [6] or catalytic oxidation of axial hydroxyl groups [7] generally provides low yields of the desired keto glycosides. The former method has the disadvantage of producing a very complex mixture of products, involving further oxidation in the molecule. Such doubly oxidized products are extremely labile and readily give rise to rearrangement products.[8] The separation of the desired product from the starting material and by-products severely limits the preparation of large amounts of oxidized glycosides by such methods. Catalytic oxidation suffers from the disadvantage of the necessity for a specific substrate. Since oxidation of axial secondary alcohols takes place most readily the starting material must possess the correct stereochemistry. This may involve a rather complex synthetic problem. Keto glycosides having an axial secondary hydroxyl or

* Described in part in an earlier communication.[1]

** Present address: Department of Chemistry, Simon Fraser University, Burnaby 2, B. C., Canada.

a primary hydroxyl group free are difficult to synthesize due to the ease of oxidation of these functional groups.

The introduction of blocking groups to limit the extent of oxidation necessitates rather specific properties for such a group. The trityl function [9] and various acetals [10,11] have been used in this role. The problem lies in the choice of a group stable to the oxidative conditions and yet easily removed by reagents which do not degrade the sensitive keto glycoside. Methods employing basic conditions are unsuitable due to the lability of keto glycosides in the presence of base.[8] Phenylboronate esters [12] have properties which suggest their value in the synthesis of keto glycosides. The ability of phenylboronic acid to complex with 1,3-diaxial or 1,2-*cis* glycols enables the simultaneous blocking of two specific sites in a typical glycoside in a stereospecific manner. The biggest advantage offered by phenylboronate esters is their ease of removal and thus the subsequent release of keto glycoside under mild conditions.

This ready hydrolysis of the protective ester necessitates use of a non-hydroxylic mild oxidative reagent and for this reason, the modified Pfitzner-Moffatt reagent [13] described by Albright and Goldman [14] was chosen. That the phenylboronate esters of methyl xylopyranosides were stable under such conditions was indicated by the very rapid drop in the optical rotation at 313 mμ during the oxidation. This suggests a negative Cotton effect [15] and thus a 1-C conformation (necessary for the phenylboronate ester) for the newly formed compound. The sign of rotation was opposite to that obtained from the free keto glycoside in solution, indicating that the molecule had reverted to the more stable C-1 conformation. The high yield of keto product also precludes loss of the protective group during the reaction as the keto glycoside is easily oxidized further. Thus treatment of the oxidation mixture with water not only stops the oxidation but releases the unsubstituted keto glycoside from its protective ester.

The remaining problem of isolation of the keto glycosides from oxidation mixtures was resolved through the application of bisulphite resin columns. That strongly basic anion exchange resins in the bisulphite form still possess the reactivity to form bisulphite addition complexes with aldehydes and ketones has been well demonstrated by Samuelson.[16] Thus an aqueous oxidation mixture, which had been extracted with chloroform to remove excess acetic anhydride and presumably phenylboronic acid was passed through a column of Dowex 1 (HSO_3^-) maintained at 15° by a cooling water jacket. The resin was then washed with water at that temperature until no dimethylsulphoxide was evident in the eluate. Finally, the column was raised to an appropriate temperature which was related to the stability of the bisulphite complex and the pure keto glycoside was eluted with either water or aqueous acetone. Experiments are described in which the known anomeric methyl-D-xylopyranoside-2,4-phenylboronates [17] are oxidized to their 3-keto derivatives and isolated as the pure methyl-D-*erythro*-pentopyranosid-3-uloses. The extension of this work to the 1,6-anhydro-β-D-glucopyranose system and the formation of 1,6-anhydro-β-D-*ribo*-hexopyranos-3-ulose will be described later.[18]

Recently, lyophilization has been used to isolate keto sugars from dimethylsulphoxide-acetic anhydride oxidation mixtures [19] and this will no

doubt extend the use of this oxidation to compounds which cannot be purified on bisulphite resin columns. Elution of keto sugars from bisulphite resin is dependent on the stability of the bisulphite adduct. α-D-Pyranosid-3-uloses are most easily removed due to the 1,3-diaxial interaction of the glycosidic group with addition complex at C-3.[20] β-D-Pyranosid-3-uloses are next easiest to remove with 2- and 4-keto pyranosides being still more difficult. Keto furanosides such as methyl α- and β-D-*threo*-pentofuranosidulose and 6-aldehydo glycosides could not be eluted from the resin with either hot (80°) water or high acetone (75 %) concentrations,[18] this being due to the stability of their bisulphite adducts. The observation that D-glucose is eluted from a bisulphite column more slowly than D-fructose has led to an effective procedure for the separation of ketoses from aldoses [18] on a preparative scale. Such observations indicate that the greater the stability of the bisulphite addition complex as indicated by bisulphite buffer electrophoresis,[21] the more difficult the elution of keto sugars from bisulphite columns becomes.

The utility of bisulphite resin columns for the isolation of keto glycosides from complex oxidation mixtures was indicated by the isolation of crystalline methyl α-D-*ribo*-hexopyranosid-3-ulose from the oxidation of methyl α-D-glucopyranoside. A similar treatment of sucrose yielded α-D-*ribo*-hexopyranosid-3-ulose-β-D-fructofuranoside in low yield. These latter preparations were designed as examples that illustrate the intrinsic properties of the bisulphite column technique rather than as preparative methods for the production of the particular keto sugars.

EXPERIMENTAL

Methyl α-D-erythro-pentopyranosid-3-ulose. Methyl α-D-xylopyranoside 2,4-phenylboronate [17] (1.29 g) was dissolved in a mixture of acetic anhydride (20 ml) and dimethylsulphoxide (30 ml) and the solution was kept at 40° for 75 min. The solution was then poured into a mixture of chloroform (100 ml) and water (100 ml). The aqueous phase was extracted and the chloroform layer discarded. The aqueous phase was extracted twice further with chloroform (2 × 100 ml). The aqueous phase was then percolated slowly (3 ml/min) through a column of Dowex 1 × 4 (HSO_3^-) (200 ml). The column was then washed with cold water (300 ml) and finally a warm (45°) solution of 10 % acetone in water was used as eluent. The eluent containing keto sugar, as detected by spot tests on paper with *p*-anisidine hydrochloride, was passed through Dowex 50 (H^+) (10 ml) and Dowex 1 (OAc^-) (20 ml) to remove any traces of inorganic material. The eluent was evaporated to a thick syrup at a bath temperature of 35°. The syrup crystallized to give material (420 mg, 50 %) which melted at 79–82°. The product, methyl α-D-*erythro*-pentopyranosid-3-ulose, could be vacuum sublimed at 70° to give crystals melting at 80–82°, $[\alpha]_{578}^{20}$ + 191° (*c*, 1.2 in water) M_v 0.26 (pH 4.7, bisulphite electrophoresis).[20] (Found: C 44.0; H 6.1. $C_6H_{10}O_5$ requires: C 44.4; H 6.2).

Reduction of the product with sodium borohydride gave a mixture of methyl α-D-ribopyranoside and methyl α-D-xylopyranoside (24:1) as determined by GLC of the trimethylsilyl ethers.

Methyl β-D-erythro-pentopyranosid-3-ulose. This material was prepared in 80 % yield in exactly the same manner as the α-anomer using methyl β-D-xylopyranoside 2,4-phenylboronate as the starting material. The product could be sublimed at 70° to yield pure methyl β-D-*erythro*-pentopyranosid-3-ulose, m.p. 85–88°, $[\alpha]_{578}^{20}$ −77° (*c*, 1 in water). M_v 0.99. Lit. M_v 1.00.[21] (Found: C 44.4; H 6.1. $C_6H_{10}O_5$ requires: C 44.4; H 6.2).

Reduction with sodium borohydride gave a mixture of methyl β-D-ribopyranoside and methyl β-D-xylopyranoside in the ratio of 0.82:1.

Methyl α-D-ribo-hexopyranosid-3-ulose. To a solution of methyl α-D-glucopyranoside (5 g) in dimethylsulphoxide (60 ml), acetic anhydride (40 ml) was added. The solution was kept at 60° for 20 min, cooled, diluted with chloroform (200 ml) and extracted with water (200 ml). The aqueous phase was extracted twice further with chloroform (2 × 200 ml) and was passed slowly through a column of Dowex 1 (HSO_3^-) (4 × 45 cm jacketed column) at 10—12°. The resin was then washed with water (1500 ml). The bottom of the column was fitted with a small column of Dowex 3 (free base). Elution was carried out at 40° using water as the eluent and collecting fractions of 250 ml. Fractions 2—6 contained material positive to the silver nitrate-sodium hydroxide reagent and these fractions were evaporated to give a crystalline material (0.3 g., 6 %). Recrystallization from a small amount of butanone gave methyl α-D-*ribo*-hexopyranosid-3-ulose, m.p. 91—92°. $[\alpha]_D^{25}$ + 155° (*c*, 2.6 in water). M_V 0.08. Lit. M_V 0.1.[21] (Found: C 44.2; H 6.4. Calc. for $C_7H_{12}O_6$: C 43.8; H 6.3).

Reduction with sodium borohydride gave a mixture of methyl α-D-allopyranoside and methyl α-D-glucopyranoside in the ratio of 66:1.

α-D-Ribo-hexopyranosyl-3-ulose-β-D-fructofuranoside. To a solution of sucrose (2.5 g) in dimethylsulphoxide (30 ml), acetic anhydride (20 ml) was added. The solution was kept at 65° for 20 min, cooled, diluted with chloroform (100 ml) and extracted with water (100 ml). The aqueous phase was extracted twice with chloroform (2 × 100 ml) and was passed slowly through a column of Dowex 1 (HSO_3^-) (4 × 45 cm jacketed column) at 15°. The resin was then washed with water (2000 ml) at 15°. The temperature of the column was raised to 50° and a small column (2 × 7 cm) of Dowex 3 (free base) was fitted to the bottom of the large column. Using water as an eluent the fraction from 250—1000 ml was collected and evaporated to a small volume (50 ml). This solution contained the product contaminated with fructose and glucose and was refractionated on fresh bisulphite resin at 30° collecting 250 ml fractions. Fractions 1—5 contained glucose and fractions 6—11 contained keto sucrose. These fractions were evaporated at 40° to give a syrup (100 mg, 4 %) $[\alpha]_D^{23}$ + 52° (*c*, 4 in H_2O). M_V 0.1.

Although the product did not crystallize as has been reported for this material,[23] on reduction with sodium borohydride followed by hydrolysis, only glucose, allose, and fructose were indicated by paper chromatography and germanate buffer electrophoresis.[24] Methanolysis of the reduced ketosucrose followed by gas liquid chromatography of the trimethylsilyl ethers indicated that only compounds derived from allose, fructose, and glucose were present.

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