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Note

Acetylative desulfation of a glucose-6-sulfate: An oxygen isotope labelling study

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In 1950 Wolfrom and Montgomery reported[1] that carbohydrate hydrogensulfates undergo an acetylative desulfation reaction when placed in acetic anhydride containing an excess of sulfuric acid. This chemistry was reminiscent of that hypothesized by Malm et al.[2], who had invoked the acetolysis of hydrogensulfate intermediates in the sulfuric acid-catalyzed acetylation of polysaccharides.

I have observed that 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose-6-sulfate, potassium salt (**1**) is cleanly converted to 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**2**) when heated under reflux in acetic anhydride (Scheme 1). This reaction (which proceeds rapidly at 130°C but not at a measurable rate at 25°C) presents an interesting mechanistic problem: How, in the absence of hydroxylic nucleophiles or water, does the conversion of sulfate to acetate take place? Although formally similar to a transesterification reaction, this process differs from transesterifications by the absence of plausible nucleophiles and the improbability of nucleophilic attack at the sulfate sulfur[3].

I have considered two mechanisms for the desulfation: In the first case (Scheme 2), the potassium sulfate **1** reacts with acetic anhydride to give an intermediate mixed anhydride **3**, which then undergoes an electrocyclic extrusion of sulfur trioxide to yield the acetate **2**.

In the second case (Scheme 3), reversible unimolecular extrusion of sulfur trioxide from **1** would produce a low concentration of alkoxide **4**, which could be irreversibly trapped with acetic anhydride to give product **2**. A mechanism of this type has been proposed by Benkovic and coworkers for the aqueous hydrolysis of *p*-nitrophenyl hydrogensulfate[4,5]; more recently Williams and co-workers have interpreted similar processes in terms of an open or "exploded" transition state[6,7].

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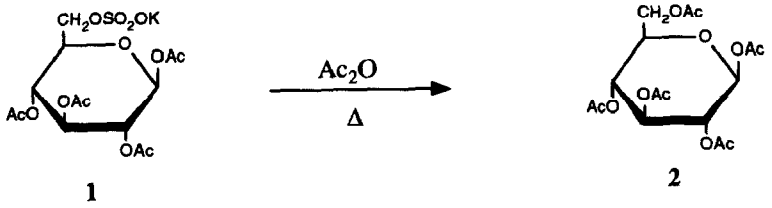
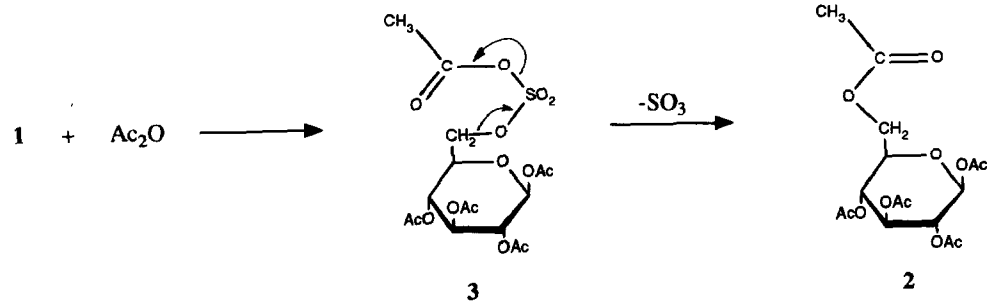


1 2

Scheme 1.

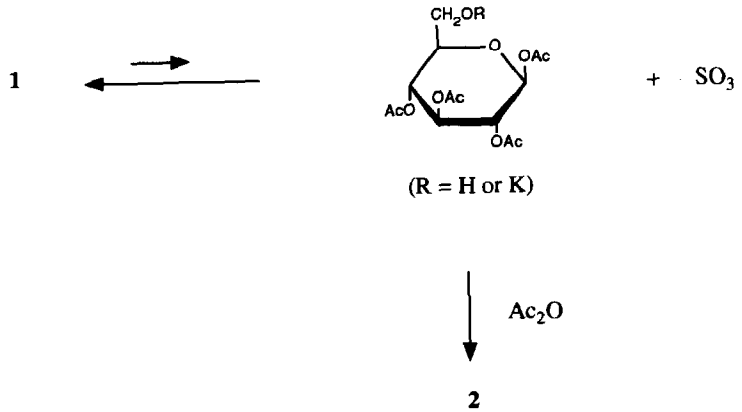
Case 1:



3 2

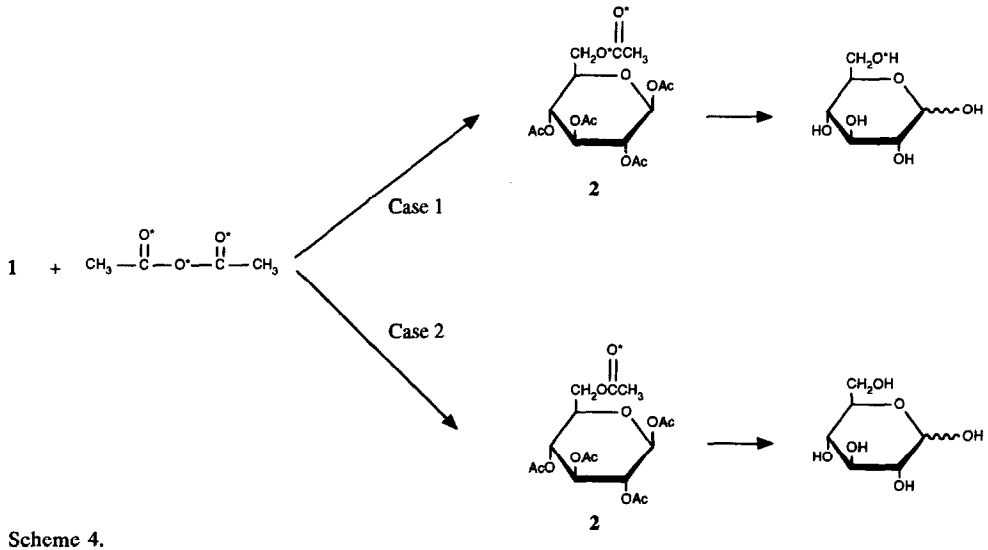
Scheme 2.

Case 2:



2

Scheme 3.



Scheme 4.

(It is not implied that free SO_3 would exist under our reaction conditions as it would be complexed to either the acyl or sugar oxygens.)

The use of ^{18}O isotope labelling provides a method of distinguishing between these two mechanisms. If unlabelled **1** were allowed to react with labelled acetic anhydride, the mechanism of the first case would lead to a product bearing ^{18}O label on the *glucose* O-6, whereas the second mechanism would place the label on the Ac-6 *acyl* oxygen. Removal of the acetyls by methanolysis (Scheme 4) and mass spectral analysis of the resulting glucose would thus permit determination of the labeling pattern.

It was found that glucose that was recovered from the (^{18}O)acetylative desulfation–deacetylation sequence (when analyzed by ammonia CIMS of the per-trimethylsilyl ether) incorporated less than 2% ^{18}O. The isotopic purity of the (^{18}O)acetic anhydride was such that the electrocyclic mechanism would have led to 86% incorporation in the glucose. I therefore conclude that the electrocyclic sulfur trioxide expulsion pathway is not important in this reaction, and that acetylative desulfation in acetic anhydride probably proceeds via production of a low level of alcohol by extrusion of sulfur trioxide from the starting material. This pathway may also account for the results reported in refs. 1 and 2 as well, and may be pertinent in the case of carbohydrate hydrogensulfate hydrolysis[10].

EXPERIMENTAL

General. — Mass spectrometry experiments were performed on Finnigan MAT TSQ 70 or SSQ 70 spectrometers equipped with PDP-11 data systems. Theoretical isotope ratios were calculated using the VG-70 OPUS data system.

1,2,3,4-Tetra-O-acetyl-β-D-glucopyranose-6-sulfate, potassium salt (**1**).—The method of Guiseley and Ruoff[8] was used to prepare the corresponding barium salt, which was too insoluble for use in the present research. An Amberlyst IR-120 (H^+ form) column (2 × 30 cm) was prepared in water and washed with 5% aq KCl until the effluent was neutral. The column was then washed with distilled water until no further chloride was eluted ($AgNO_3$ test). A solution of 0.30 g (0.53 mmol) of the barium salt in 100 mL of water was passed through the column, followed by an additional 100 mL of water. The effluent was evaporated under vacuum at 45°C to provide crude **1** as a syrup. The crude product was dissolved in ~5 mL of boiling 90% EtOH and set aside to crystallize at 25°C. After 24 h, [filtration gave 0.147 g of **1** as fine white needles of mp 170–175°C (dec)]. A second recrystallization raised the decomposition temperature to 220–222°C. The product was a non-hygroscopic monohydrate. IR (mull): 1745, 1220 br, 1110, 1095, 1072, 1040, 1003, 932, and 800 br cm^{-1}. ^1H NMR (D_2O, 300 MHz): δ 5.93 (d, 1 H), 5.44 (t, 1 H), 5.21 (m, 2 H), 4.18–4.28 (br m, 3 H), and 2.05–2.20 (4 partially resolved lines, 12 H). ^{13}C NMR (D_2O, 75 MHz): δ 180.1, 179.4, 179.6, 178.8, 98.7, 80.1, 79.2, 77.5, 74.8, 72.6, 27.3, and 27.1–27.2 (3 partially resolved peaks). FABMS (negative ion): *m/z* 427 (calcd 427). Anal. Calcd for $C_{14}H_{19}KO_{13}S \cdot H_2O$: C, 34.7; H, 4.37. Found: C, 34.8; H, 4.10.

Reaction of **1** *with acetic anhydride*: A mixture of 15 mg (0.035 mmol) of salt **1** and 2 mL of acetic anhydride was heated under reflux for 2 h. The mixture was then stripped of volatiles under high vacuum, and the residue was analysed by NMR spectroscopy, which disclosed the presence of β-D-glucose pentaacetate (**2**) and an additional peak attributed to acetic acid (the presence of acetic acid was probably caused by protonation of a KOAc byproduct with traces of HCl in the $CDCl_3$ solvent). The product was recovered from the NMR sample by evaporation, and the solid was recrystallized from EtOH to give 11 mg (80%) of **2**; mp 108–109°C (lit. 109–111°C).

(^{18}O)Acetic anhydride.—A modification of the procedure of Berg-Nielsen and Skattebol[9] was used: a 250-mL flask was carefully dried, purged with Ar, and charged with 3.60 g (0.056 mol) of (^{18}O)acetic acid (MSD Isotopes, lot 2653-p) and 74 mL of dry CH_2Cl_2. The mixture was stirred, and 7.74 g (0.061 mol) of oxalyl chloride was added via syringe. The mixture was stirred under reflux for 3 h, cooled to 25°C, and purged of HCl by bubbling Ar into the liquid via a syringe for 30 min. To the mixture was then added 5.0 g (0.069 mol) of sodium (^{18}O)acetate (Cambridge Isotope Labs, lot CA-008). The resulting slurry was stirred for 18 h at 25°C and filtered through a pad of Celite. Most of the CH_2Cl_2 was removed from the filtrate by distillation through a 15-cm Vigreaux column. When the still pot residue was ~8 mL, the Vigreaux column was replaced with a short-path still, and distillation was continued. After removal of additional CH_2Cl_2, fractions were obtained of bp 112–126°C (1.4 g, mostly acetic acid), 126–132°C (1.3 g, 12 mol% acetic acid, 88% acetic anhydride by NMR spectroscopy), and 132–138°C (1.6 g, 97.4% acetic anhydride, 2.6% acetic acid by NMR spectroscopy).

The amount of ^{18}O enrichment of the last fraction was determined by GLC–MS employing ammonia chemical ionization. The sample was chromatographed on a J&W 30-m, 0.32-mm i.d., 0.25-μ film thickness DB-5 column that was temperature programmed from 50 to 200°C at 15 °C/min. The carrier gas was He at a linear velocity of 30 cm/s as determined with air at 100°C. Injection and transfer lines were set at 250 and 275°C, respectively. The source temperature was 150°C, and the ammonia pressure was adjusted to give a ratio of approximately 5:1 for m/z 18 to 35. The spectrometer was scanned from m/z 116–130 in 0.1 s to monitor the ammonium acetate cluster of the acetic anhydride. Care was taken to ensure that the data system was not saturated with the signal. All scans (usually 30) within the GLC peak were averaged for each of two repetitions; both experiments gave 85.5% ^{18}O incorporation in the acetic anhydride. The cluster comprised 5% relative intensity m/z 122 (1 ^{18}O), 32% m/z 124 (2 ^{18}O), and 63% m/z 126 (3 ^{18}O). The contribution of ^{13}C to the various cluster peaks was calculated to be less than 1% and was ignored.

Reaction of **1** *with (*^{18}O*)acetic anhydride.*—A solution of 0.050 g (0.107 mmol) of salt **1** in 1.0 mL of (^{18}O)acetic anhydride was protected from moisture and heated under reflux for 3 h. Analysis of the resulting mixture (TLC) showed the absence of **1** and formation of pentaacetate **2** as the sole product. Evaporation of volatiles under a high vacuum left a solid residue whose NMR spectrum showed the presence of only **2**. This solid (10 mg) was added to 2.5 mL of freshly prepared abs MeOH and treated with 2 drops of a solution of 50 mg of Na metal in 60 mL of abs MeOH. After 1 h, TLC analysis indicated that traces of **2** remained and an additional drop of methoxide solution was added. After standing 18 h, the methanolysis mixture was neutralized by addition of 1 drop of acetic acid and stripped of solvents under vacuum. The dry residue was taken up in 1 mL of dry pyridine and treated with 0.5 mL of Regisil® silylation reagent (Regis Chemical Co.). The resulting solution was shown by GLC to contain a ~1:1 mixture of α- and β-D-glucose pertrimethylsilyl ethers.

The glucose pertrimethylsilyl ether solution was analysed by GLC–MS as described above in the case of labelled acetic anhydride. The spectrometer was scanned from m/z 550–570 in 0.1 s. The sample concentration was adjusted to given between 100 000 and 1 000 000 counts in the total ion chromatogram with a multiplier setting between 1200 and 1800 V. Analysis of control (unlabelled) α- and β-D-glucose trimethylsilyl ethers (10 repetitions, averaged) gave M + 2/M isotope ratios of 0.26 and 0.27, respectively; the theoretical value for this ratio was 0.298. The isotope-labelled sample, analyzed as above and corrected for natural abundance, gave 1.7 and 2.0% ^{18}O incorporation for the two anomers.

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