Hydrolysis of *O*-Isopropenyl Glucopyranosides Involves *C*-Protonation and Alkenyl Ether Cleavage and Exhibits a Kinetic Influence of Anomeric Configuration

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Summary: Hydrolysis of *O*-isopropenyl α-glucopyranoside occurs by *C*-protonation and alkenyl ether (not glycosidic) C–O bond cleavage and proceeds in 10 mM salicylate buffer, at pH 3.0 and 25 °C, 4.5 times faster than the similar hydrolysis of its β anomer.

As part of an investigation of *O*-isopropenyl glycosides[1,2] as potential substrates for enzyme-catalyzed and electrophile-mediated transglycosylations,[3] we have examined the mechanism and rates of hydrolysis of *O*-isopropenyl α-D-glucopyranoside, **1**, and its β anomer, **2**. Before our work, it was not apparent whether a vinyl glycoside would undergo hydrolysis of the glycosidic (acetal) or vinyl ether C–O bond. Also not obvious was whether or not the anomeric configuration would influence the mechanism or rates of hydrolysis of **1** and **2**. We report here that, at pH 3.0 and 25 °C, both **1** and **2** hydrolyze exclusively by irreversible, rate-limiting *C*-protonation followed by alkenyl ether cleavage, not glycosidic bond cleavage (Scheme 1). Furthermore, compounds **1** and **2** exhibit an influence of anomeric configuration on their rates of hydrolysis.[4] In salicylate buffer, the α anomer, **1**, hydrolyzes 4.5 times faster than the β anomer, **2**. Compounds **1** and **2** are the first alkyl vinyl acetals reported to hydrolyze exclusively by cleavage of the vinyl ether C–O bond.[5]

Compound **1** was prepared from penta-*O*-pivaloyl-β-D-glucopyranose[6] in three steps (Scheme 2). Acetolysis of the pentapivaloate gave only the α-acetoxy compound, which was recrystallized (mp 144–145 °C) before further use. Methylidenation by dimethyltitanocene[7] proved to be sterically selective, allowing clean conversion of the anomeric acetoxy group into the isopropenyl ether in the presence of the pivaloate esters. Compound **2** was prepared by reaction of tetra-*O*-acetylglucopyranosyl bromide with diacetonylmercury,[1,8] followed by deacetylation (Scheme 3). The anomeric configurations of **1** and **2** were determined by ^{1}H NMR.[9,10] Additionally, α-glucosidase from yeast catalyzed the hydrolysis of **1** but not **2**, and β-glucosidase from almonds catalyzed the hydrolysis of **2** but not **1**.

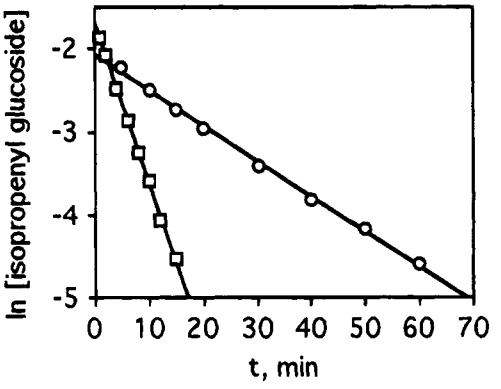

Figure 1. First-order hydrolysis of *O*-isopropenyl α-glucopyranoside **1** (□) and *O*-isopropenyl β-glucopyranoside **2** (○) in 10 mM sodium salicylate buffer, pH 3.0, at 25 °C.

Compounds **1** and **2** (approximately 12 mM initial concentrations) were hydrolyzed in 10 mM sodium salicylate buffer, pH 3.0, at 25 °C. Aliquots of the hydrolysis reactions were removed periodically, quenched with 50 mM Tris buffer, pH 7.5,[11] and assayed enzymatically for glucose.[12] The measured rates of hydrolysis were first order with respect to each isopropenyl glucoside for more than 4 half-lives (Figure 1). Rate constants for hydrolysis of **1** and **2** are 3.21×10^{-3} and 7.11×10^{-4} s^{-1}, respectively. These rates are 10–100 times greater than the rates of hydrolysis of alkyl and aryl glucosides at pH 0.0 and 60 °C.[13]

When the hydrolysis of **1** (28 mM initial concentration) was performed in buffered D_2O and observed by ^{1}H NMR, the initially formed product was exclusively α-glucopyranose (Scheme 1). Disappearance of the signal for the anomeric proton of **1** was accompanied by the appearance of the anomeric proton signal for α-glucopyranose (δ 5.23, J = 3.7 Hz) but not of that for β-glucopyranose (δ 4.65, J

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(9) Compound **1**: mp 157–159 °C; ^{1}H NMR (D_2O/TSP, 250 MHz) δ 5.45 (d, J = 3.7 Hz, 1 H), 4.35 (d, J = 1.6 Hz, 1 H), 4.22 (br, 1 H), 3.76–3.85 (m, 3 H), 3.61–3.71 (m, 2 H), 3.46 (t, J = 9.4 Hz, 1 H), 1.88 (br s, 3 H). Anal. Calcd for $C_9H_{16}O_6$: C, 49.09; H, 7.32. Found: C, 49.15; H, 7.54.

(10) Compound **2**: mp 137–138 °C; ^{1}H NMR (D_2O/TSP, 250 MHz) δ 4.94 (d, J = 7.8 Hz, 1 H), 4.29 (d, J = 2.1 Hz, 1 H), 4.23 (br, 1 H), 3.92 (dd, J = 2.0, 12.4 Hz, 1 H), 3.72 (dd, J = 5.6, 12.4 Hz, 1 H), 3.54–3.61 (m, 2 H), 3.39–3.47 (m, 2 H) , 1.88 (d, J = 0.54 Hz, 3 H). Anal. Calcd for $C_9H_{16}O_6$: C, 49.09; H, 7.32. Found: C, 48.92; H, 7.50.

(11) No detectable hydrolysis of **1** or **2** occurred during 8 h in 50 mM Tris buffer, pH 7.5.

(12) The procedure used was an adaptation of the methods reported by (a) Kunst, A.; Draeger, B.; Ziegenhorn, J. In *Methods of Enzymatic Analysis*; 3rd ed.; Bergmeyer, H. U., Bergmeyer, J., and Grassl, M., Eds.; Verlag Chemie: Deerfield Beach, FL, 1984; Vol. 6; pp 163–172. (b) Bergmeyer, H. U.; Grassl, M.; Walter, H.-E. *Ibid.* 1983; Vol. 2; pp 222–223.

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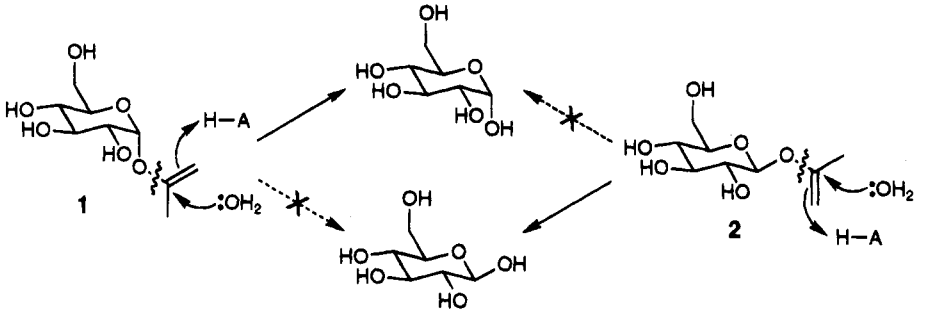
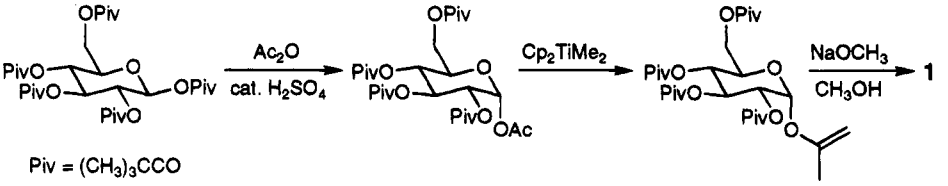
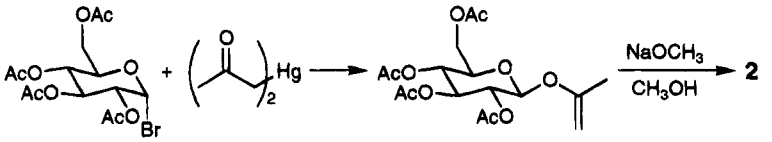
Scheme 1. Mechanism and Stereochemical Outcome of the Hydrolysis of *O*-Isopropenyl α- and β-Glucopyranosides, 1 and 2, in 10 mM Sodium Salicylate Buffer, pH 3.0, at 25 °C



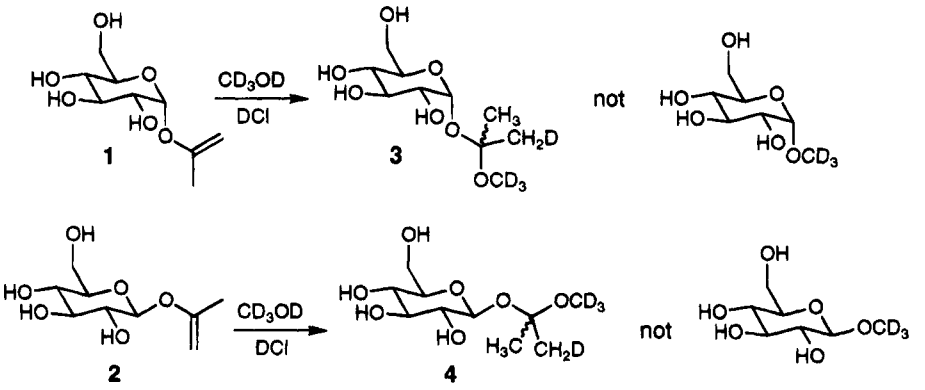
Scheme 2. Synthesis of *O*-Isopropenyl α-D-Glucopyranoside, 1



Scheme 3. Synthesis of *O*-Isopropenyl β-D-Glucopyranoside, 2



Scheme 4. Products Formed by Reaction of *O*-Isopropenyl α- and β-Glucopyranosides, 1 and 2, with Methanol-d_4 Containing 1 mM DCl



= 7.9 Hz). Similarly, hydrolysis of **2** generated β-glucopyranose exclusively as the initially formed product. No exchange of deuterium for the vinyl protons of **1** or **2** was observed. The rates of hydrolysis of **1** and **2** in buffered D_2O were more than six times slower than those measured under essentially identical conditions in H_2O. Furthermore, initial experiments with other carboxylic acid buffers show that the rates of hydrolysis increase with increasing concentration of buffer and that the rate constants for buffer-catalyzed hydrolysis increase with the acidity of the acid component of the buffer. Both the large normal solvent deuterium isotope effect and the influence of buffer concentration and acid strength on the rates of hydrolysis suggest rate-limiting *C*-protonation of the substrates by the general acid component of the buffer. This feature is consistent with alkenyl ether C–O bond cleavage, not glycosidic (acetal) C–O bond cleavage.[14]

Reaction of **1** and **2** in methanol-d_4 containing 1 mM DCl[15] generated glucosyl methyl acetonides **3** and **4**, respectively, and not methyl glucopyranosides as the products (Scheme 4). When monitored by ^{1}H NMR, reaction of **1** led to the disappearance of signals for the anomeric (δ 5.20, J = 3.6 Hz), vinyl (δ 4.26 and 3.97), and methyl (δ 1.76) protons of **1** and the appearance of new anomeric (δ 5.01, J = 3.7 Hz) and methyl (δ 1.21) resonances consistent with the structure of **3**. Neither the anomeric signal for methyl α- or β-glucopyranoside (δ 4.56, J = 3.7 Hz, and δ 4.06, J = 7.7, respectively) nor the proton resonance of acetone (δ 2.06) was observed. The newly formed methyl peak at δ 1.21 integrated for five protons relative to the anomeric doublet at δ 5.01 and had an upfield shoulder, consistent with the incorporation of one solvent-derived deuteron into one of two magnetically equivalent methyl groups. The reaction of

(14) Kresge, A. J.; Chiang, Y. *J. Chem. Soc. B* **1967**, 58–61.

(15) DCl in methanol-d_4 was prepared by adding benzoyl chloride to methanol-d_4.

2 in methanol-d_4 proceeded similarly. The disappearance of ^{1}H NMR signals for the anomeric (δ 4.62, J = 7.4 Hz), vinyl (δ 4.13 and 3.97), and methyl (δ 1.75) protons of **2** was accompanied by the appearance of new anomeric (δ 4.38, J = 7.7 Hz) and methyl (δ 1.20) resonances consistent with the structure of **4**. Neither the anomeric signal for methyl α- or β-glucopyranoside nor the proton resonance of acetone was observed. Again, no exchange of deuterium for the vinyl protons of **1** or **2** was observed. Consistent with the rates of hydrolysis, the methanolysis of **1** occurred 2.6 times faster than that of **2** ($k = 7.6 \times 10^{-3}$ and 2.9×10^{-3} s^{-1}, respectively).

All experimental evidence indicates that the hydrolyses of **1** and **2** involve irreversible, rate-limiting *C*-protonation, followed by alkenyl ether C–O bond cleavage, not glycosidic C–O bond cleavage. The fact that **1** hydrolyzes more rapidly than **2** suggests that **1** is kinetically more basic than **2**. The greater lability of **1** cannot be attributed to steric strain in the reactants since *O*-isopropyl β-glucopyranoside hydrolyzes 2.1 times faster than the corresponding α-glucopyranoside.[16] The cause for the greater kinetic basicity of **1** cannot be steric accessiblity or dipole–dipole interactions since these should make **2** more reactive than **1**. The β-isopropenyloxy group is sterically more accessible to general acids than the α-isopropenyloxy group, and dipole–dipole repulsion between exocyclic and endocyclic C–O bonds is greater in **2** than in **1**. Any factor which reduces conjugation between the glycosidic oxygen and the carbon–carbon double bond of the isopropenyl group, however, could be responsible for attenuating the reactivity of **2**. The endocyclic n–σ^* interaction[4] that is present in **1** but absent in **2** could be such a factor. In **1**, overlap of the nonbonding electrons on the pyranose ring oxygen with the σ^* orbital of the glycosidic C–O bond should lengthen the glycosidic C–O bond and shorten the endocyclic C(1)–O bond,[17] thereby raising the energy of the C(1)–O(ring) σ^* orbital. Both the longer glycosidic C–O bond and the higher energy of the C(1)–O(ring) σ^* orbital would reduce the exocyclic n–σ^* interaction between the glycosidic oxygen and the C(1)–O(ring) bond. The reduced exocyclic n–σ^* interaction in **1** would leave the isopropenyloxy group of **1** kinetically more basic than that of **2**, which has no endocyclic n–σ^* interaction opposing the exocyclic n–σ^* interaction. The reactivities of **1** and **2** may thus constitute a novel approach to determining the influence of anomeric configuration on the electronic state of the glycosidic oxygen.

Alternatively, either conformational differences between **1** and **2** or differences in solvation could influence the degree of conjugation between the glycosidic oxygens and the alkenyl double bonds. Increased hydrogen bonding to water may reduce the conjugation of the β-(isopropenyloxy) group of **2**. Calculations have predicted that, in water, the anomeric substituents of β-glucopyranose[18] and β-2-methoxytetrahydropyrans[19,20] are more heavily solvated than those of the corresponding α anomers. Experiments to distinguish between the effects of n–σ^* overlap, conformation, and solvation on the reactivities of **1** and **2** are underway in our laboratory.

Acknowledgment. We thank Alfredo Castro for providing an initial sample of **2** and for measuring the rate of hydrolysis of **2** catalyzed by β-glucosidase. This work has been supported, in part, by the National Science Foundation (CHE 90-19078), the Petroleum Research Fund (ACS-PRF 25395-G1), and the University of Delaware Research Foundation.

Supplementary Material Available: Experimental procedures (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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