Exocyclic and Endocyclic Cleavage of Pyranosides in Both Methanol and Water Detected by a Novel Probe

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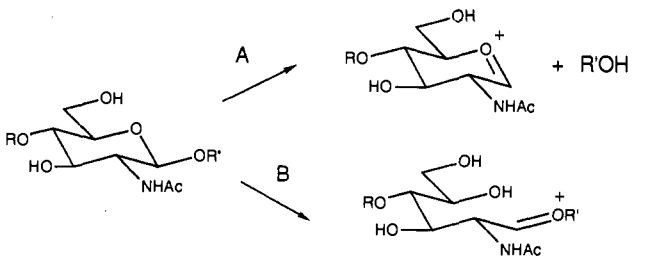
The mechanism of acetal hydrolysis, as it relates to fundamental saccharide transformations such as glycosyl transfer, has been the subject of much investigation.[1] Lysozyme is a paradigmatic enzyme for this transformation;[2] therefore, the mechanism of β-acetal hydrolysis by lysozyme is of particular interest. Previous kinetic studies,[3] model building,[4] and recent crystallographic data for the enzyme[5] have been the basis for the proposed exocyclic cleavage mechanism involving cyclic oxocarbenium ion intermediates (path A, Scheme 1). Kinetic isotope effects in the specific acid-catalyzed hydrolysis of pyranosides also lend support to this mechanism.[6] Exocyclic cleavage of β-anomers, however, does not follow the theory of stereoelectronic control unless conformational strain is introduced into the pyranose ring.[7] Based on molecular dynamics calculations, Karplus[8] has proposed an alternative mechanism that does follow stereoelectronic control.[9] His proposed mechanism involves endocyclic cleavage to give an acyclic oxocarbenium ion (path B, Scheme 1). Support for this suggestion is the observation of endocyclic cleavage in furanosides,[10] and specific acid-catalyzed solvolysis[11] as well as Lewis acid mediated ring opening[12] of glycopyranosides. In aqueous media, however, evidence of endocyclic cleavage of pyranosides is lacking. Presented herein are the results of specific acid-catalyzed methanolysis and hydrolysis studies of a pyranoside which provide definitive support for the existence of endocyclic cleavage.

To distinguish between the endo- and exocyclic cleavage pathways a pyranoside probe (**1**-d_2) was designed containing an intramolecular nucleophile with an effective molarity of 30 000–50 000 M.[13] The key feature of this probe is the differing symmetry of the oxocarbenium ions generated by each cleavage pathway (Scheme 2). Upon solvolysis of **1**, the asymmetric oxocarbenium ion **2** cannot be trapped because of the insufficient length of the appended nucleophile, and thus only the α- and β-methyl acetals **3** and **4** can be formed. However, the symmetric oxocarbenium ion **5** generated from endocyclic cleavage yields not only **3** and **4** but also the deuterium-scrambled counterparts

Scheme 1



6 and **7**. In addition, by reverse of the ring opening, deuterium can be incorporated into the pyran ring of the starting material (**8**). Therefore, endocyclic cleavage results in a portion of deuterium-scrambled products (**6** and **7**) and deuterium-scrambled starting material (**8**), whereas exocyclic cleavage results in no deuterium scrambling.[14]

Endocyclic cleavage was indicated by the appearance of ^{1}H NMR resonances for the CH_2OH appendage during solvolysis. After solvolysis, the α-anomer products (**3** and **6**), the β-anomer products (**4** and **7**), and the recovered starting material (**1** and **8**) were separated by HPLC. The 500-MHz ^{1}H NMR spectra in $CDCl_3$ were then obtained, and the CH_2OH resonances between 3.3 and 3.6 ppm integrated relative to the bridgehead and anomeric resonances. To determine the experimental limits for such integrations, a series of aliquots of **1**-h_2 were added to **1**-d_2. A plot of the relative integral versus percent **1**-h_2 was linear for additions above 3% **1**-h_2. This indicated a lower limit of 3% deuterium scrambling for accurate quantitation, yet as little as 2% could be visually detected. Since either the h_2 or d_2 appendages in **5** may trap the oxocarbenium ion, the experimental limit of 3% scrambling corresponds to a lower quantitation limit of 6% endocyclic cleavage.

The methanolysis (CH_3OH-d_4) of β-**1**-d_2 was performed with a catalytic amount of DCl and monitored with a 300-MHz ^{1}H NMR spectrometer. As the products are also acetals subject to further solvolysis, the reaction was stopped at 30% completion by the addition of triethylamine. In the formation of α-methyl acetal products (**3** and **6**, Figure 1A), 14% endocyclic cleavage was observed. For the mixture of β-methyl acetal products (**4** and **7**), approximately 30% endocyclic cleavage was detected.[15a] Hence, the ratio of endocyclic to exocyclic pathways for the formation of α and β products is different.[15b] Scrambling was detected but not quantifiable in the recovered starting material.

As a measure of the importance of stereoelectronic considerations for the proposed pathways of solvolysis of acetals,[9] and as a means to verify that isotopic scrambling does not arise from secondary solvolysis of the α-anomer products (**3** and **6**), the methanolysis of an α-isopropyl probe (α-**1**-d_2) was conducted. Based upon the theory of stereoelectronic control, α-anomers are predicted to proceed via the exocyclic mechanism.[9] Indeed, the products from α-**1**-d_2 showed negligible scrambling even after 100% completion of primary solvolysis.

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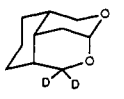
(14) Compound **1** is a substituted *cis*-decalin derivative and therefore is conformationally mobile. (See ref 14a.) Molecular mechanics predicts that the alternate conformation is 6 kcal/mol higher in energy. The alternate conformation, however, is an α-acetal and would yield **9**, which should accumulate (as observed by others in similar systems). (See ref 14b.) (a) Mann, B. E. *J. Magn. Reson.* **1976**, *21*, 17. (b) Beaulieu, N.; Dickinson, R. A.; Deslongchamps, P. *Can. J. Chem.* **1980**, *58*, 2531.



(15) (a) The β-methyl product is not shown in Figure 1 since it is a minor product, and its ^{1}H NMR spectra cannot be obtained in a manner consistent with the quantitation limit experiment. (b) The reason for this difference is currently under investigation.

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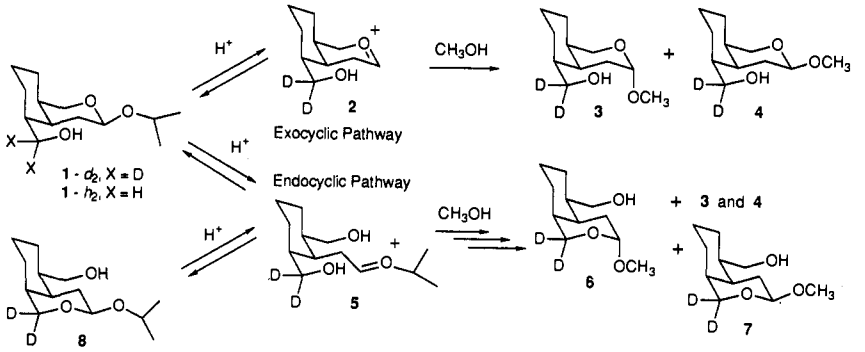
Scheme 2. Exocyclic Cleavage Cannot Scramble the Isotopic Label of **1**, Whereas the Label Can Scramble into Both the Starting Material and Products for Endocyclic Cleavage



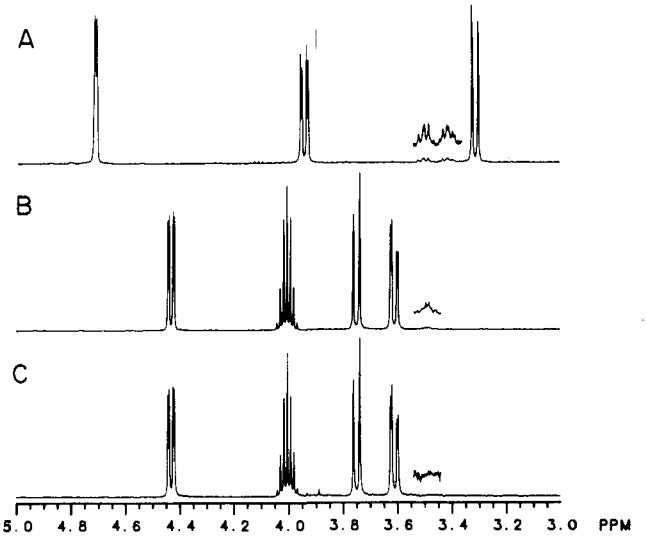


Figure 1. (A) Isolated α-methyl anomer of **1** from methanolysis. The resonances at 3.4 and 3.5 ppm indicate deuterium scrambling. (B) Recovered starting material from the hydrolysis of **1**. The resonance at 3.5 ppm indicates deuterium scrambling. (C) β-**1**-d_2 before solvolysis.

Endocyclic cleavage in water was also detected by the use of probe β-**1**-d_2. Analysis of the products in a similar manner to that in the methanolysis studies, however, provides no valuable information because mutarotation generates scrambling in the hemiacetal product at a significantly greater rate than hydrolysis. Instead, the DCl catalyzed hydrolysis of β-**1**-d_2 in D_2O[16] was allowed to proceed to 55% completion, and the nonhydrolyzed material (**1** and **8**) was isolated and analyzed. It indicated incorporation of the deuterium-labeled appendage into the pyran ring corresponding to 8% endocyclic cleavage (Figure 1B). The 8% represents only a minimum for endocyclic cleavage, whereas the actual value can be detected only as the reaction approaches completion.

In methanol, less endocyclic cleavage is indicated in the recovered starting material than in the α-products, which may be indicative of nucleophilic assistance[17a] or solvent cage effects.[17b]

(16) The D_2O contained 10% CH_3CN-d_3 to solubilize **1**.

If the oxocarbenium ion **5** is solvent-equilibrated, the high effective molarity of the appendage should cause scrambling of the isotopic label of the starting material faster than product formation. This is not observed, therefore as discussed previously,[18] structures **2** and **5** may more resemble transition states than full oxocarbenium ion intermediates in methanol.[19] Furthermore, in water the extrapolated lifetimes of the oxocarbenium ions (10^{-12}–10^{-15} s)[20] also indicate that the intermediates may be too unstable to exist.

Endocyclic cleavage has not been detected in aqueous media previously. The unique design of probe **1**, however, allows for the detection of endocyclic cleavage in aqueous media for two reasons. First is the generation of the symmetric intermediate, which upon the reverse of the initial ring opening introduces deuterium into the pyran ring. Second, because of its high effective concentration, the appendage should yield intramolecular trapping near 10^4 faster than water.[13] This effective molarity is the same as that involved in the reverse of the ring opening.

In summary, for the methanolysis of β-pyranoside **1**, 14–30% endocyclic cleavage has been detected, and a minimum 8% has been observed in water. In contrast, the α-anomer of **1** shows negligible endocyclic cleavage. Although endocyclic cleavage appears to be a minor pathway, the mechanism proposed by Karplus for lysozyme is operative in nonenzymatic hydrolysis.

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Supplementary Material Available: Synthetic route to **1** and chemical characterization of **1** (2 pages). This material is contained in many 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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