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Supplementary Material Available: Tables of complete CAD spectra of CH_2=$CHCO^+$ and $C_3H_3O^+$, the fourth field-free spectrum of $\mathbf{1}^{\cdot+}$, and the calculated harmonic vibrational frequencies (3 pages). Ordering information is given on any current masthead page.

Linkage Position Determination in Lithium-Cationized Disaccharides: Tandem Mass Spectrometry and Semiempirical Calculations

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Abstract: Fast atom bombardment ionization in combination with tandem mass spectrometry of lithiated disaccharides is used to differentiate the linkage position of five isomeric sugars. Labeling experiments in combination with product and precursor ion scans indicate that reducing ring opening occurs followed by two-, three-, and four-carbon cleavage. Carbonyl migration via keto–enol tautomerization is postulated as an isomerization that occurs during CID, thus enabling specific cleavages for each of the different isomers. Semiempirical calculations of both the hemiacetal and hydroxy aldehyde forms support experimental data that indicate that the lithium ion is pentacoordinate between the two rings.

Introduction

Fast atom bombardment (FAB) ionization combined with tandem mass spectrometry (MS/MS) has been exemplified as a method for determining both sequence and branching patterns of derivatized[1-3] and underivatized[4-6] oligosaccharides. Extensions of these studies include investigations of alkali-metal attachment to disaccharides and larger oligomers via different ionization techniques including laser desorption[7-11] as well as FAB[12,13] in an effort to better understand differences in fragmentation and stability of ions when alkali metals replace hydrogen in the cationic precursor. In a previous study, we reported preliminary data on linkage position determination when the dilithiated precursor of trisaccharides and tetrasaccharides was activated.[13] In order to better understand this phenomenon, we have investigated one isomer and subsequently applied this information to the other four isomeric disaccharides.

In this study, we show how the linkage position of different isomeric disaccharides can be distinguished when the monolithiated precursor undergoes collision-induced dissociation (CID) after FAB ionization. Deuterium- and ^{18}O-labeling studies were used to unambiguously determine where glycosidic cleavage is occurring. These labeling studies in conjunction with precursor and product ion scans show that specific reducing ring cleavage takes place when the monolithiated precursor of the various isomeric disaccharides undergoes CID. Since protonated molecular ions do not exhibit ring cleavage with FAB ionization and low-energy CID, the question of lithium-induced cleavage is addressed. Semiempirical calculations are also provided in an effort to better determine the position and extent of coordination of lithium to the molecule. The isomer gentiobiose (1,6-linked disaccharide) is investigated in detail, and mechanisms of fragmentation are postulated that are supported by experimental results and semiempirical calculations. These data are then applied to the 1,4, 1,3, 1,2, and 1,1 isomers, and the mechanism of fragmentation that gives information on linkage position is outlined.

Experimental Section

Collision-Induced Dissociation. Product ion spectra were generated from CID of the monolithiated molecular ions $(M + Li)^+$ transmitted into the quadrupole collision cell (q) of a BEqQ geometry instrument with 30–110-eV collision energy (laboratory frame of reference). Argon gas pressure ($1-2 \times 10^{-6}$ Torr) was measured with an ion gauge near the quadrupole collision cell. Low-energy CID spectra were obtained by scanning the mass analyzer up in mass from 50–400 u in 15–20 s, and 5–10 scans were acquired under data system control in the "MCA" (multichannel analyzer) mode. The mass scale of the quadrupole analyzer (Q) was calibrated by using low- and high-mass ions obtained from CID of the cluster ions of glycerol. Unit mass resolution was achieved for analysis of the isotopically labeled compounds, and was confirmed by obtaining resolution of the product ions at m/z 278 and 279 u of leucine-enkephalin.

Precursor Ion Scans. Precursor ion spectra were generated by scanning the magnet (B) while setting Q to pass only the mass of the product ion. This type of scan was again acquired under data system control with the mass range 100–500 u used for the front end scan.

Compounds. All disaccharides were purchased from Sigma Chemical Co. The deuterated compound gentiobiose-d_8 was prepared by dissolving 1 mg of disaccharide in 400 μL of D_2O (99.9% D, Cambridge Isotope Laboratories), and then lyophilizing the sample. This procedure was repeated three times, and then the sample was dissolved in 100 μL of D_2O to use as the stock solution. The ^{18}O-labeled gentiobiose compound was prepared by adding 0.5 μL of acetyl chloride to 1 mg of gentiobiose, dissolved in 100 μL of $^{18}OH_2$ (MSD, 97%, normalized for hydrogen

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Table I. Tabulated Product Ion Spectral Data of Different Linked Disaccharides

compound	linkage	product ions produced (m/z, neutral)					
		331, $-H_2O$	289, $-C_2H_4O_2$	259, $-C_3H_6O_3$	229, $-C_4H_8O_4$	187, $-C_6H_{10}O_5$	169, $-C_6H_{12}O_6$
gentiobiose	β-1,6	X	X	X	X	X	X
melibiose	α-1,6	X	X	X	X	X	X
maltose	β-1,4	X	X			X	X
lactose	α-1,4	X	X			X	X
laminarbiose	β-1,3	X		X		X	X
nigerose	α-1,3	X		X		X	X
sophorose	α-1,2				X	X	X
trehalose	α-1,1					X	X

versus deuterium content). This solution was kept in a sealed vial and allowed to sit for 3 weeks in a dessicator, after which time it was lyophilized, and the sample redissolved in 100 μL of $^{18}OH_2$ for use as a stock solution.

All analysis samples were prepared by combining 5 μL of the stock disaccharide solution (1–2 mg in 100 μL of water [D_2O, for the deuterated sample] or DMSO), 0.5 μL of lithium carbonate solution (0.07 M in water (Li_2CO_3 was vacuum dried, and D_2O solvent was used for analysis of the deuterated compound)), and 4–5 μL of dithiothreitol/dithioerythritol (DTT/DTE) matrix (3:1 mixture; deuterated DTT/DTE was prepared by lyophilizing three times with D_2O, as described above, to use with the deuterated compound). Approximately 10 nmol of sample was used for each analysis.

Semiempirical Calculations. Modified neglect of differential overlap (MNDO) calculations were performed by using a general-purpose semiempirical molecular orbital package (MOPAC) written by James J. P. Stewart,[14] using a CAChe (computer-aided chemistry) calculation package developed by Tektronix corporation.[15] The starting geometry of the disaccharide was obtained from the Cambridge Structural Database.[16] A lithium atom was introduced within coordination distance of several oxygens of the disaccharide, and torsion angles were modified to accommodate the lithium ion in different locations, by using the graphics portion of the CAChe system package. Calculations were performed with the disaccharide in both the hemiacetal and the open hydroxy aldehyde forms. The calculations were carried out by the standard MNDO programs[17] based on the restricted Hartree–Fock (RHF) method. Geometries were optimized in internal coordinates and were terminated when Herberts test was satisfied in the Broyden-Fletcher-Goldfarb-Shanno method (BFGS). All optimizations were terminated when the change in energy on successive iterations was less than 0.3 kcal/mol. The heats of formation were compared for different calculated structures, in order to determine the most stable structure(s) of the monolithiated gentiobiose molecular ion. Cartesian coordinates for all structures are available in the supplementary material. Further structure details are described in the Results and Discussion.

Results and Discussion

Table I lists the product ions produced ($m/z > 160$) from CID of the monolithiated $(M + Li)^+$ precursor ions of a number of isomeric disaccharides. The pattern of product ions produced is different for each different type of linkage, demonstrating the ability to use this methodology for establishing the linkage position in unknown saccharides.[13] These results, along with several experiments performed on isotopically labeled compounds discussed below, suggest possible mechanisms for formation of the different ions observed. We have also performed MNDO calculations of the monolithiated ion of gentiobiose in both the hemiacetal and hydroxy aldehyde form, in order to determine the most stable structure(s) of the precursor ion.

Monolithiated gentiobiose, and other 1,6-linked lithiated disaccharides produce six major product ions of $m/z > 160$, as indicated in Table I. The ion at m/z 331 is due to loss of water, and the other fragment ions may arise from the precursor ion as shown in Figure 1. Note that, in Figure 1a, the product ions are shown to result from cleavage of the reducing sugar ring. Results obtained from labeling experiments, which are discussed below, support this hypothesis. Similarly, Figure 1b indicates that the product ions at m/z 187 and 169 could result from cleavage on either side of the glycosidic oxygen atom, with lithium ion retention on either side. Determination of which fragment ion retains the charge is also discussed below.

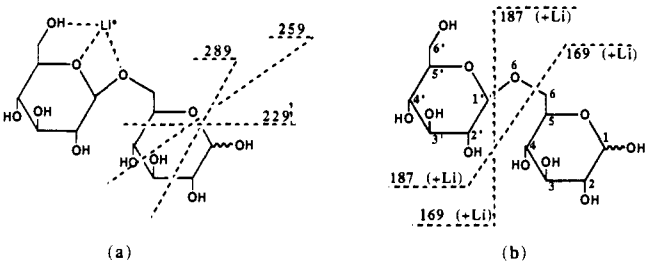


Figure 1. Fragmentation of monolithiated gentiobiose results from (a) cleavage of the reducing sugar ring; and (b) cleavage of glycosidic linkage.

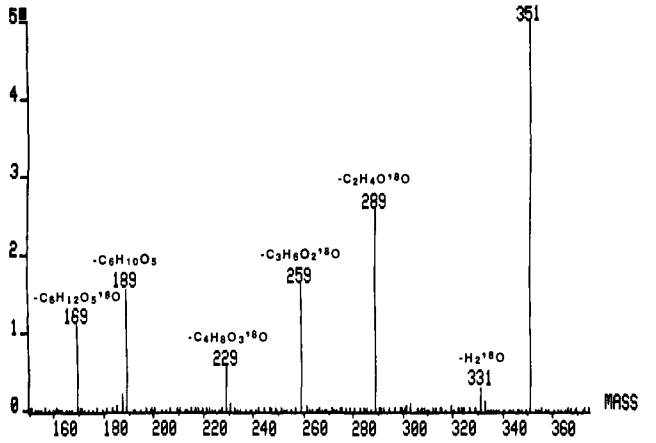


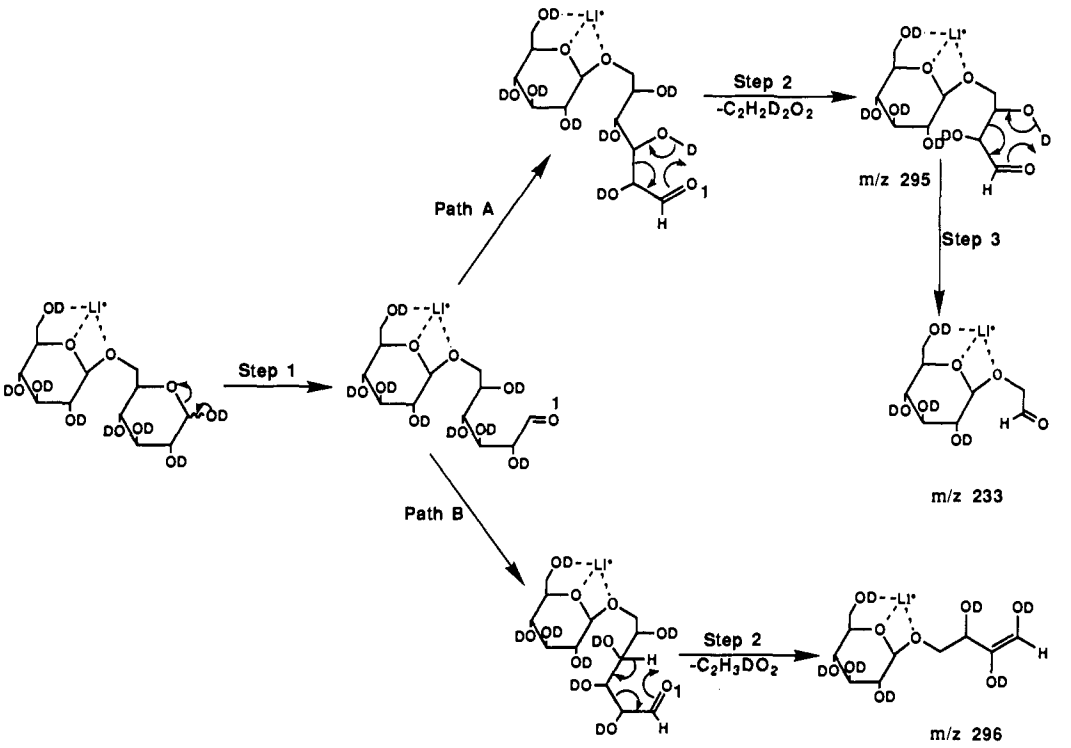
Figure 2. CID product ion spectrum of $[M + Li]^+$ from anomeric ^{18}O-labeled gentiobiose.

Labeling Studies. Gentiobiose was specifically labeled with ^{18}O at the anomeric position, in order to determine if O1 is contained in the loss of water, and to establish whether the losses forming ions at m/z 289, 259, and 229 are from reducing or nonreducing ring cleavage. The CID product ion spectrum from monolithiated ^{18}O-labeled gentiobiose is shown in Figure 2. The ion at m/z 331 is formed from the loss of ^{18}O-labeled water, indicating that the majority of the water lost results from cleavage of the C1–O1 bond. The ions at m/z 289, 259, and 229 also involve loss of the ^{18}O-labeled portion of the molecule, and support reducing ring cleavage of the molecule, as shown in Figure 1a. Glycosidic cleavage of monolithiated ^{18}O gentiobiose produces major fragment ions at m/z 189 and 169. Clearly, the ion at m/z 189 is the same as that occurring at m/z 187 in the unlabeled gentiobiose, except that it now contains an ^{18}O atom. Therefore, the glycosidic bond must break predominantly between C1′ and O6, and the two fragment ions arise from partitioning of the lithium ion on either side of this cleavage.

The CID product ion spectrum of gentiobiose, in which all of the hydroxyl protons are replaced by deuterium, was also acquired (Figure 3). This experiment provided information regarding the mechanism of the bond cleavages occurring in these systems. The ions at m/z 337 and 172 are due to loss of D_2O and $C_6D_5H_7O_6$,

(14) Stewart, J. J.; Frank, J. Seiler Research Labs, United States Air Force Academy, Colorado Springs, CO 80840, version 5.10.
(15) Tektronix Corp., version 2.2.
(16) Cambridge Crystallographic Data Center, 1990.
(17) (a) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899–4907. (b) MNDOC: Thiel, W. *QCPE* **1982**, *438* (2), 63.

Scheme I



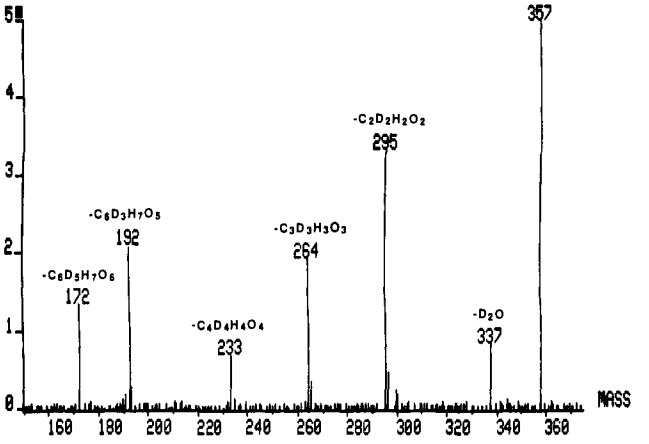
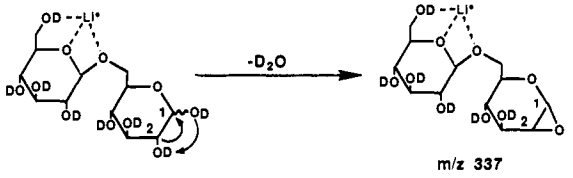


Figure 3. CID product ion spectrum of $[M + Li]^+$ from deuterated gentiobiose.

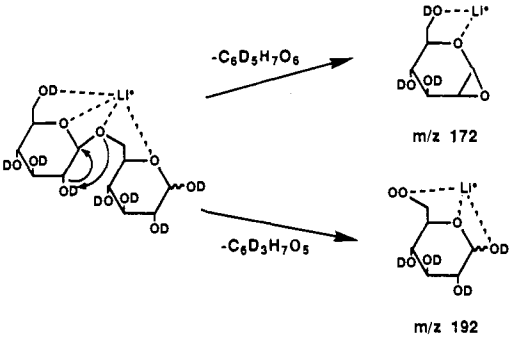
respectively, and this indicates that a deuterium atom, rather than a hydrogen, has been transferred to the reducing ring species upon fragmentation. This implies that the mechanism of cleavage involves abstracting a hydroxyl proton rather than a carbon proton. Similarly, the reducing ring cleavages also proceed by abstraction of a hydroxyl proton, as discussed below.

On the basis of the information described above, a mechanism for water loss is proposed in eq 1. The data presented in Table I support this mechanism, because water is not lost from either the 1,2- or 1,1-linked disaccharides. The 1,1-linked disaccharide does not lose water, because the hydroxyl group at C1 is tied up in the glycosidic bond. The absence of water loss from the 1,2-linked disaccharide suggests that the second hydroxyl proton originates from the hydroxyl group at C2. A similar mechanism has been suggested by Djerassi et al. to explain the water loss from 1,2-diol radical cations.[18]



(1)

An attractive mechanism for glycosidic bond cleavage is one similar to that proposed for water loss, and is shown in eq 2. The product ion spectrum of gentiobiose-d_8 (Figure 3) is consistent with this proposed mechanism, because the reducing ring abstracts a deuterium rather than a hydrogen from the nonreducing ring. However, it remains to protect the O2′ oxygen, in order to determine whether this mechanism is operative. There may be several pathways for effecting glycosidic bond cleavage.

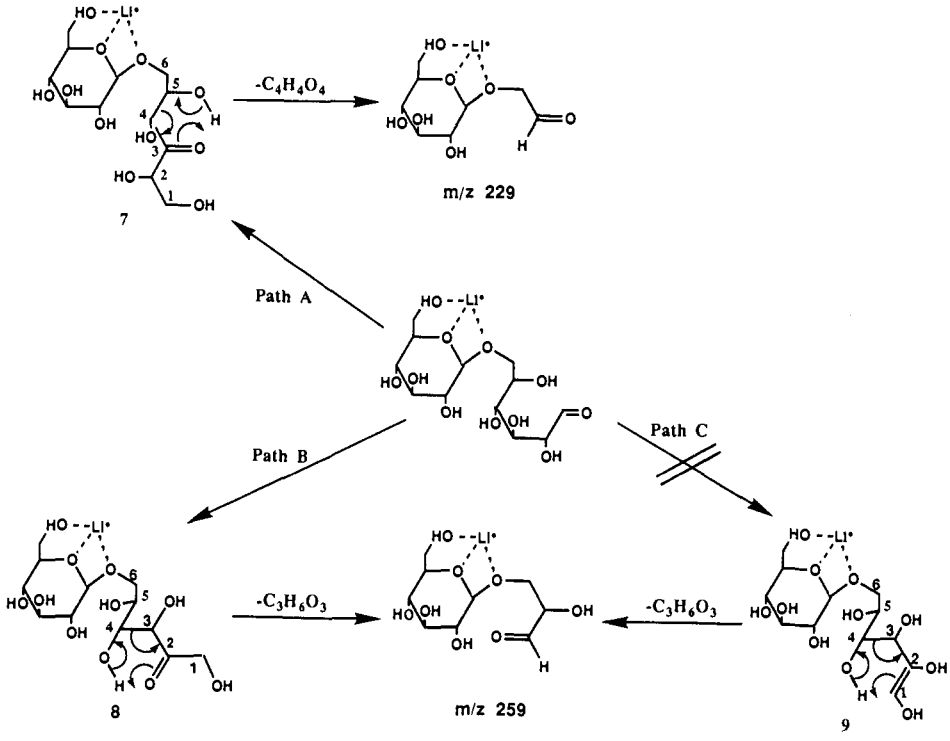


(2)

Reducing Ring Fragmentation. At this stage of interpretation, reducing ring fragmentation has been established; however, further analysis of all of the presented data provides insight into the mechanisms of the two-bond ring-breaking reactions shown in Figure 1a. Since simultaneous breaking of two bonds is difficult to envision, a stepwise mechanism seems appropriate for these reactions. The first step appears to be opening of the reducing ring during CID to form the acyclic hydroxy aldehyde species (step 1, Scheme I). This step has also been proposed by Cotter et al. to occur by rapid heating from infrared laser irradiation during IR laser desorption mass spectrometry (IR LDMS) of oligosaccharides.[8] This postulated step is supported by the absence of any product ions of m/z 289, 259, and/or 229 when trehalose, a 1,1-linked disaccharide, is the precursor ion (Table I). The anomeric oxygen is protected in this compound, which prevents the necessary ring-opening step, thereby precluding the formation of product ions due to reducing ring cleavage (possible lithium-catalyzed ring opening is discussed further on).

(18) Kossanyi, J.; Morizur, J. P.; Furth, B.; Wiemann, J.; Duffield, A. M.; Djerassi, C. *Org. Mass Spectrom.* **1968**, *1*, 777–789.

Scheme II



Once the sugar is in the hydroxy aldehyde form, there are two possible mechanisms that can account for the two-carbon fragment loss. These pathways have been described by Cotter et al. for the protonated form of the 1,4-linked isomer cellobiose[8] and are outlined in Scheme I for the deuterium-labeled 1,6-linked isomer gentiobiose. Both paths A (retro-aldol) and B (retro-ene) are reasonable since they invoke a stable six-membered transition state. The retro-aldol path can be distinguished from the retro-ene path by whether a hydrogen or deuterium is transferred to the carbonyl group in the two-carbon fragmentation from deuterated gentiobiose (Scheme I). The CID spectrum of deuterated gentiobiose shows an ion at m/z 295 (not m/z 296), which is due to the loss of $C_2H_2D_2O_2$. This experiment thus supports the retro-aldol mechanism for the two-carbon loss.

Four- and Three-Carbon Losses. The four-carbon fragment loss might easily occur via another retro-aldol reaction from the product ion of m/z 295 (step 3, Scheme I). Cotter and co-workers also observe a similar four-carbon fragmentation for protonated cellobiose and attribute it to this consecutive mechanism.[8] In our lithium system, however, a precursor ion scan of m/z 229 (four-carbon loss from unlabeled monolithiated gentiobiose) indicates that both $[M + Li]^+$ (m/z 349) and $[M + Li - C_2H_4O_2]^+$ (m/z 289) are precursors of this ion (Figure 4). In fact, the ion of m/z 289 is not very abundant in comparison to m/z 349, suggesting that the majority of the product forming at m/z 229 comes directly from the monolithiated molecular ion. This four-carbon fragmentation (Scheme II, path A) can occur from the lithiated molecular ion by first inducing carbonyl migration to C3 via keto–enol tautomerization. (Two consecutive hydrogen 1,2-migrations as well as two hydrogen 1,4- and hydrogen 1,3-rearrangements have been reported previously.[19]) Although we have no direct evidence of this intermediate (**7**), we have additional supporting evidence of hydrogen 1,2- and hydrogen 1,3-rearrangements, presented below.

The loss of the three-carbon neutral producing a product ion at m/z 259 can be explained if one again considers carbonyl migration prior to bond cleavage (Scheme II, path B). Note that, via this mechanism, the oxygens at C2 and C4 are both involved in the transition state to promote a three-carbon fragmentation. This mechanism is supported by the data from the 1,2- and 1,4-linked disaccharides, which do not produce an ion at m/z 259 under CID conditions. These isomers have protected oxygens at the C2 and C4 positions, respectively, and thus cannot participate in the mechanism outlined in path B. One might argue that path C could also produce a three-carbon loss. However, if this were the case, the 1,2-linked isomer should have fragmented via this pathway, producing an ion at m/z 259, which it does not. We believe that collision activation assists in isomerization of the precursor ion to intermediates such as **7** and **8**, so that three- and four-carbon cleavage is observed, as well as two-carbon fragmentation.

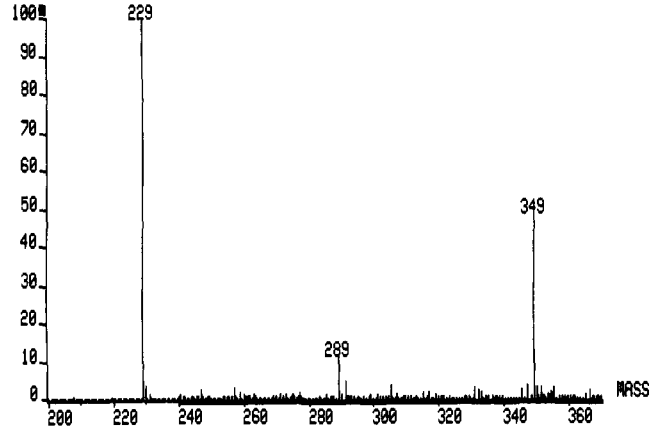


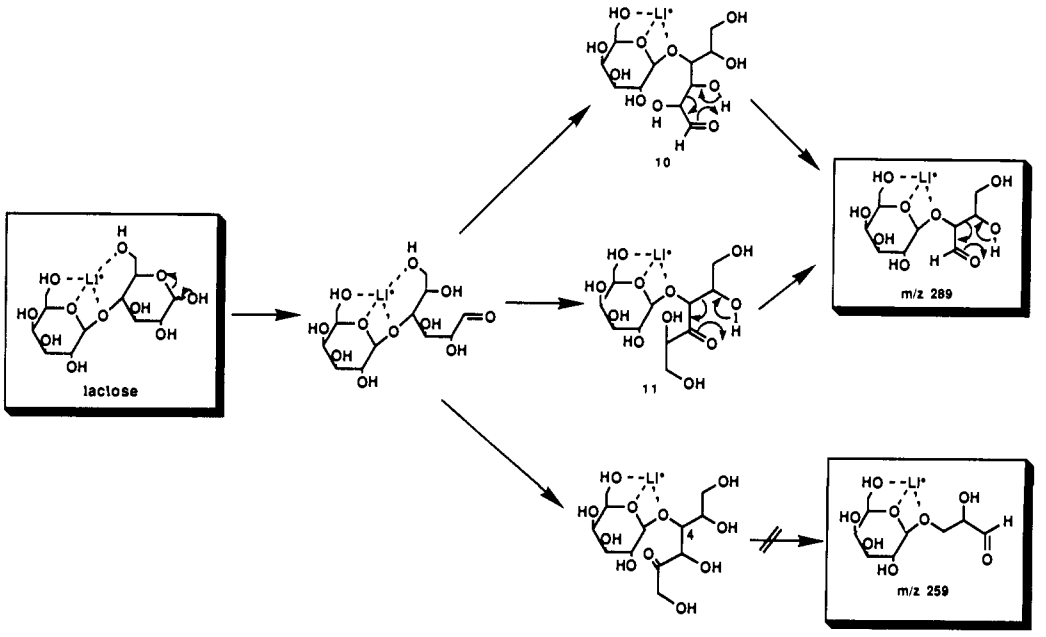
Figure 4. Precursor ion spectrum of m/z 229, which corresponds to four-carbon loss from monolithiated gentiobiose.

An important point to note is that when the product ion spectrum is acquired after the sample has been bombarded by Cs^+ ions for 5–7 min, the intensity of product ions in the spectrum increases by a factor of 5. Furthermore, the ions due to reducing ring fragmentation (m/z 289, 259, 229) increase by a greater amount, relative to the ions due to glycosidic cleavage (m/z 187 and 169).[20] We interpret this as an indication that some portion

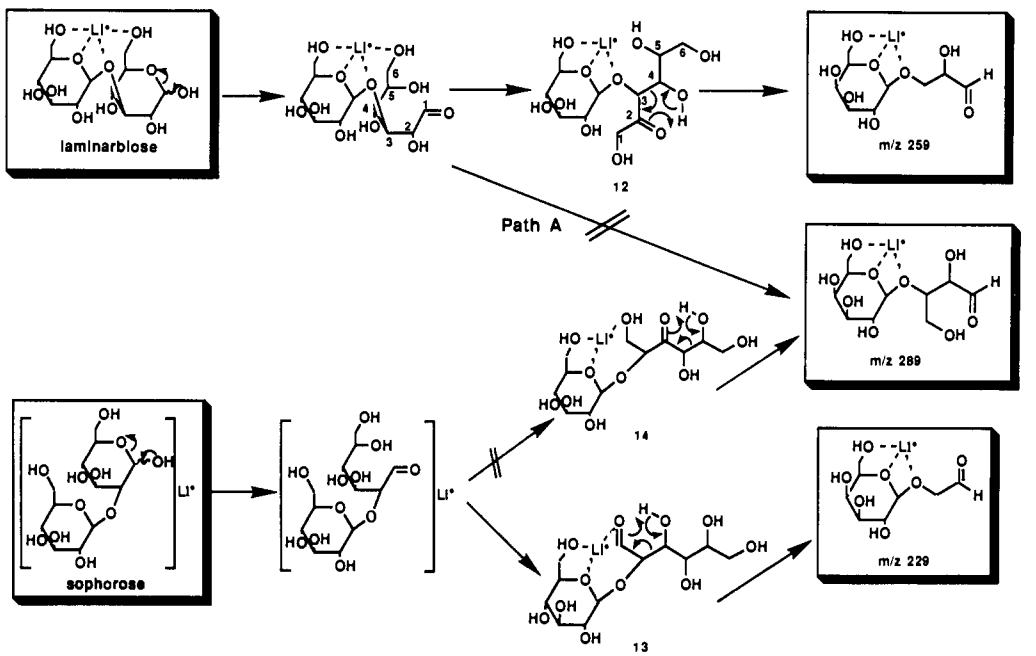
(19) Levsen, K.; Schwarz, H. *Mass Spectrom. Rev.* **1983**, *2*, 77–148.

(20) There is no difference in the low-resolution FAB spectrum over a 29-min acquisition period; i.e., only glycosidic bond cleavage is observed, and there is no indication of reducing ring cleavage taking place when ions are detected at the second detector prior to collision activation.

Scheme III



Scheme IV



of the precursor ion population is changing. Either it has gained more internal energy, or a different isomeric structure has been formed, which more readily gives rise to the indicated product ions. This phenomenon might be explained by possible radiative damage to the sample during FAB, such that more of the hemiacetal structure is converted to the hydroxy aldehyde form via ring opening and that subsequent two-, three-, and four-carbon cleavage is induced upon activation. We are currently investigating ways of measuring the precursor ion energy distribution as well as determining if the time frame of the experiment plays an important role in energy uptake and fragmentation.

Effect of Linkage Position on Ring Cleavage. On the basis of the above mechanistic analysis, it is possible to explain how the linkage position affects the formation of reducing sugar ring fragments. Scheme III shows the fragmentation pathways available to lactose, a 1,4-linked disaccharide. The product ion at m/z 289 could arise either from the open form of the precursor ion (**10**), or from the isomer where the carbonyl group has been transposed to C3 (**11**). As explained earlier, the three-carbon fragmentation to form an ion at m/z 259 is not observed, because the C4 oxygen is protected. Also, contiguous four-carbon fragmentation to form an ion at m/z 229 is not possible, due to the linkage position.

Scheme IV depicts the fragmentation pathways available from laminarbiose (1,3-linkage) and sophorose (1,2-linkage). Laminaribiose, and other 1,3-linked disaccharides are incapable of fragmenting via a retro-aldol reaction when the carbonyl group is located at C1, because the oxygen at C3 is protected (path A). Therefore, no two-carbon loss is observed. Upon transposition of the carbonyl group to C2 (**12**), three-carbon fragmentation is possible. Four-carbon fragmentation is not observed, because there is no contiguous four-carbon chain to be lost. Deuterium labeling of laminarbiose and the subsequent CID experiment also resulted in a three-carbon chain loss corresponding to $C_3H_4O_3D_2$. These results substantiate our hypothesis of keto–enol tautomerization since loss of the above neutral could only occur after transposition of the carbonyl group of the C2 position.

Finally, sophorose produces the ion at m/z 229 by four-carbon fragmentation from the hydroxy aldehyde form (**13**). Three-carbon fragmentation is not possible, because the oxygen at C2

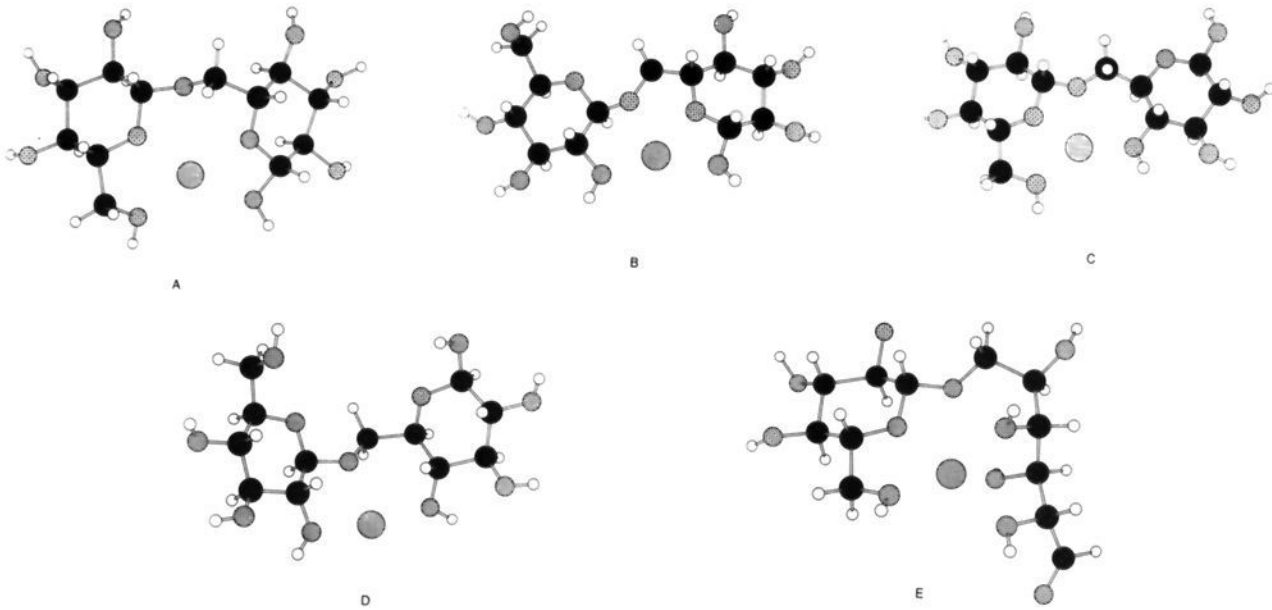


Figure 5. (A–E) show structures of lithiated gentiobiose generated from MNDO calculations together with respective heats of formation. ΔH_f = (A) −406 kcal; (B) −398 kcal; (C) −400 kcal; (D) −390 kcal; (E) −399 kcal. In (E), the reducing ring has been opened into the hydroxy aldehyde form.

is protected. Note that isomerization of the carbonyl group to C3 (**14**) could result in two-carbon fragmentation, which is not observed from the monolithiated molecular ion. However, two-carbon fragmentation is observed from the dilithiated molecular ion $(M + 2Li - H)^+$,[13] so the isomerization to **14** appears to be inhibited in the monolithiated case. Perhaps the carbonyl migration to C3 (**14**) requires proceeding through an intermediate in which the carbonyl group is located at C2, which is not possible in sophorose.

Since ring opening appears to be essential for reducing ring cleavage, it is not surprising that the 1,1 isomer produces no two-, three-, or four-carbon losses. Since the glycosidic oxygen is bonded to the anomeric carbon, ring opening is clearly inhibited.

Role of Lithium. The fragmentation mechanisms described above do not necessarily involve lithium in the transition state, so it is not immediately obvious what role lithium plays in inducing the observed fragmentation. However, it is clear that lithium and collision activation influences the fragmentation, because product ion spectra of the corresponding protonated molecular ions of underivatized saccharides show only cleavage at the glycosidic bond, with no reducing ring fragmentation under FAB ionization conditions.[12]

In order to address the effect of lithium on the gas-phase disaccharide structures, we have performed a number of MNDO calculations of monolithiated gentiobiose. A lithium ion was positioned between two adjacent oxygens of a given sugar ring, and the input structure was optimized by the MNDO program. Whenever these starting coordinating oxygens were located near the glycosidic linkage (O1, O4, O5, O6, O2′, O5′, O6′; see Figure 1b), the program adjusted the other sugar ring position, so that its oxygen atoms also coordinated the lithium ion. The resulting calculations consistently minimized to a structure that incorporates the lithium ion between the two sugar rings, where it can be coordinated by the greatest number of oxygens. All four possible combinations of this arrangement are shown in Figure 5A–D, along with the resultant heats of formation for these structures. Generally, these structures exhibit a trend in increasing stability for an increasing number of lithium-coordinating oxygens. If the starting structure contains lithium coordinated by any one of the oxygens far removed from the glycosidic linkage (O2, O3, O3′, O4′ in Figure 1b), the greatest number of possible coordinating oxygens is only two. These latter structures result in heats of formation ranging from −381 to −382 kcal, and are ca. 20 kcal less stable than those that position lithium at the glycosidic linkage. Therefore, the most probable lithium disaccharide structure is one in which the lithium bridges the two sugar rings.

We have also calculated several structures in which the reducing ring has been opened into the hydroxy aldehyde form. All of the structures calculated so far have been slightly higher in energy than the closed ring form. The least stable of these is only ca. 12 kcal less stable than those shown in Figure 5A–D. One of these results in a heat of formation of −399 kcal (Figure 5E), very close to the heats of formation for the hemiacetal. Of particular interest is the fact that the lithium ion is still coordinating between the two sugar units, even in this open ring form. Of upmost importance in interpreting these results is the understanding that we do not as yet know the internal energy of the FAB derived ions. Our calculated heats of formation are all relatively close and could easily be within the energy distribution range of the source derived ions. However, we have clearly shown that collision activation, and subsequent additional deposition of internal energy and/or isomerization, is critical for reducing ring cleavage. The calculations are a preliminary attempt at supporting our experimental data and thus enable us to envision where the lithium ion is most likely to reside.[21] The calculations are "accurate" to the extent that lithium has been previously shown to exist in a planar tetracoordinate and pentacoordinate environment with oxygen,[22] and parameterization of the program for cationic species appears internally consistent with the limited data thus far generated.[23]

The most stable calculated structures of lithiated gentiobiose are supported by the CID data, and they suggest a possible interpretation for lithium's role in the experiments outlined herein. The fact that the lithium ion is partitioned between both sides of the molecule upon glycosidic cleavage indicates that the experimental structure is similar to the calculated structure, with lithium coordinated between the two sugar rings. This mode of coordination can effectively strengthen the glycosidic linkage, so that reducing ring fragmentation can be observed. As described above, the ring fragmentation is essential for producing the structurally informative product ions. Lithium has been shown to play a similar role in preventing α-aryl 1,2-diols from fragmenting, so that the molecular ion $[M + Li]^+$ can be observed in FABMS.[24] We postulate that CID spectra of the protonated

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disaccharides do not produce the desired reducing ring fragmentation, because the proton cannot bridge the two sugars as well as lithium. Therefore, the protonated dimer falls apart in preference to fragmenting on the reducing ring. By this interpretation, lithium is indirectly inducing the ring fragmentation, by stabilizing the dimeric structure. Although lithium does not appear to be directly involved in the fragmentation mechanism, it is not inconceivable that the observed ring cleavage may be an example of remote site fragmentation.[25,26]

Other possible ways in which lithium can affect the fragmentation of these disaccharides are that it can induce ring opening of the hemiacetal to the hydroxy aldehyde, or it can assist in isomerization of the carbonyl group. Cooks has suggested that lithium may induce ring opening of sugars to the hydroxy aldehyde form,[7] but we currently have no direct evidence to support this hypothesis. It is also not clear if this ring opening takes place upon bombardment of the sample with Cs^+ ions (vide supra), or whether this occurs under CID. We believe that CID is imperative for observing reducing ring fragmentation, particularly those fragmentations that necessitate prior isomerization. However, whether lithium affects the carbonyl isomerization is unknown. We are currently exploring a means for determining the range of internal energies available to the molecular ion in these systems, for comparison to the calculated monolithiated gentiobiose structures. Furthermore, we are investigating the metastable, unimolecular decomposition pathways in these systems.

In summary, the CID product ion spectra of lithiated disaccharides result in useful linkage position information for two primary reasons. First, reducing ring fragmentation is observed, and according to our mechanistic analysis, it appears to be directly dependent on the linkage position. Furthermore, this technique would not be successful without adding lithium to the molecular ion. The lithium ion serves to stabilize the sugar dimer, so that the reducing ring fragmentation can occur. Other more subtle ways in which lithium assists in fragmentation are possible, but are not clear at this time.

Supplementary Material Available: Tables of Cartesian coordinates (10 pages). Ordering information is given on any current masthead page.

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$SiOH^+/HSiO^+$ and $SiOH^{\cdot}/HSiO^{\cdot}$: Gas-Phase Generation and Characterization. A Combined Neutralization-Reionization Mass Spectrometry and ab Initio Molecular Orbital Study†

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Abstract: Molecular orbital calculations (MP4(fc)6-311G(3df,3pd)//MP2(full)/6-311G(3df,3pd) + ZPE(MP2/full)/6-311G(3df,3pd)) are used to describe the geometries, energies, and vibrational frequencies of the four species $SiOH^+$ (1^+), $HSiO^+$ (2^+), $SiOH^{\cdot}$ ($1^{\cdot}$), and $HSiO^{\cdot}$ ($2^{\cdot}$), respectively, and the transition states for the 1,2-hydrogen migrations. In line with previous theoretical findings, all four species reside in potential minima. The most stable form invariably corresponds to the $SiOH^{+/\cdot}$ connectivity. Substantial barriers (35.3 kcal/mol for the cation and 29.5 kcal/mol for the radical) prevent facile isomerization. In line with the theoretical predictions, a combination of several mass spectrometric experiments (collisional activation and neutralization-reionization) provides evidence that all four species, which are believed to play a role in ionospheric chemistry, do indeed exist in the gas phase.

The anomalous distribution of atomic silicon in the ionosphere[1] has triggered numerous experimental and theoretical studies aimed at providing evidence for the pathways by which the depletion of ground-state Si^+ (^2P) occurs. Reactions 1-3 were proposed[2] to be responsible for the formation of protonated silicon monoxide ($SiOH^+$), which upon neutralization (eq 3) is suggested to give rise to silicon monoxide in interstellar gas clouds.[3]

$$Si^{\cdot+} + H_2O \rightarrow SiOH^+ (1^+) + H^{\cdot} \quad (1)$$

$$SiO^+ + H_2 \rightarrow SiOH^+ + H^{\cdot} \quad (2)$$

$$SiOH^+ + e^- \rightarrow SiO + H^{\cdot} \quad (3)$$

The rates for processes 1 and 2 were first measured by Fahey et al., using the flowing afterglow technique,[4] and later by Bohme

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