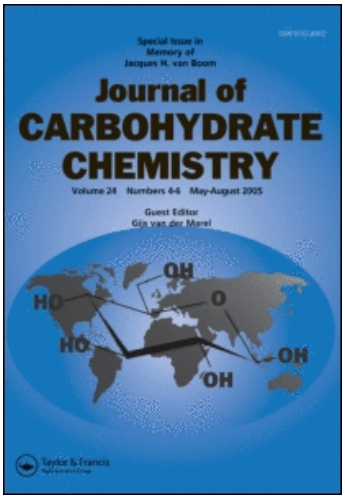


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Negative-Ion Mass Spectrometry of Carbohydrates. A Mechanistic Study of the Fragmentation Reactions of Dideoxy Sugars

Roger W. Binkley[ab]; Edith R. Binkley[b]; Shaoming Duan[a]; Michael J. S. Tevesz[a]; Witold Winnik[a]
[a] Department of Chemistry, Cleveland State University, Cleveland, Ohio [b] Center For Carbohydrate Study, Ohio

To cite this Article Binkley, Roger W. , Binkley, Edith R. , Duan, Shaoming , Tevesz, Michael J. S. and Winnik, Witold(1996) 'Negative-Ion Mass Spectrometry of Carbohydrates. A Mechanistic Study of the Fragmentation Reactions of Dideoxy Sugars', Journal of Carbohydrate Chemistry, 15: 7, 879 — 895
To link to this Article: DOI: 10.1080/07328309608005697
URL: http://dx.doi.org/10.1080/07328309608005697

J. CARBOHYDRATE CHEMISTRY, 15(7), 879-895 (1996)

NEGATIVE-ION MASS SPECTROMETRY OF CARBOHYDRATES. A MECHANISTIC STUDY OF THE FRAGMENTATION REACTIONS OF DIDEOXY SUGARS

Roger W. Binkley,[#,+] Edith R. Binkley,[+] Shaoming Duan,[#]
Michael J. S. Tevesz,[#] Witold Winnik,[#]

[#]Department of Chemistry, Cleveland State University, Cleveland Ohio 44115
[+]Center For Carbohydrate Study, 19 Hawthorne Dr., Oberlin, Ohio 44074

Received January 26, 1996 - Final Form June 5, 1996

ABSTRACT

Hydroxyl group deprotonation of the α and β anomers of methyl 3-*O*-benzyl-2,6-dideoxy-D-*arabino*-hexopyranoside (**1** and **2**) occurs readily in the gas phase to produce the corresponding anions **3** and **4**, respectively. Collisionally activated dissociation (CAD) of these anions causes fragmentation reactions that include ring opening, E2 elimination, and decarbonylation. Mechanisms for these reactions are proposed, and these mechanisms are supported by study of partially deuterated analogs of **1** and **2**.

INTRODUCTION

During the past decade considerable interest has developed in the gas phase fragmentation reactions of negative ions derived from carbohydrates.[1-21] This interest has been stimulated by an appreciation of the value of negative-ion, chemical-ionization mass spectrometry in obtaining information about carbohydrate structure. The type of information that has been obtained includes differentiation among positional and stereoisomers, determination of linkage positions, and assignment of anomeric configuration.

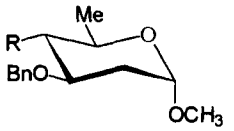
Negatively charged carbohydrates generated by chemical ionization usually experience less extensive fragmentation in the gas phase than their positively charged counterparts; in fact, these negative ions often require collisionally activated dissociation (CAD) of the pseudomolecular ion $[M-H]^-$ to produce detectable quantities of fragment ions. Since the number of fragmentation pathways available to an $[M-H]^-$ ion is limited, it is often reasonable to consider assigning structures to and reaction pathways for most, if not all, of the fragment ions produced by CAD of the $[M-H]^-$ ion. The possibility of detailed understanding of the reactions of deprotonated carbohydrates in the absence of solvent (i.e., in the gas phase) has stimulated several, pioneering mechanistic studies.[3,4,6,8,15] Even with this effort, however, understanding the fragmentation of these ions still must be regarded as in its early stages of development.

Mechanistic study of the reactions of carbohydrates is complicated especially by the fact that the position of deprotonation often is uncertain in polyhydroxy compounds.[22] One approach to solving the problem of multiple deprotonation sites is to begin study with partially protected carbohydrates. If the protection is such that only a single hydroxyl group (and no other comparably acidic group) is available, deprotonation will produce an ion of known structure. (Knowing the structure of the pseudomolecular ion is the natural "first step" in determining the fragmentation pathways.) Fragmentation of an $[M-H]^-$ ion derived from a partially protected sugar will be useful in understanding reactions of the unprotected sugar if an identifiable relationship exists between the reactivity of the protected and unprotected ions.

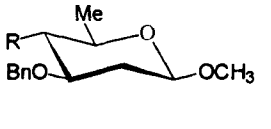
Study of ions derived from partially protected sugars to obtain information about unprotected sugars is an approach with significant potential complications. The presence of a protecting group may cause the fragmentation of the ion from a partially protected carbohydrate to be quite different from that of the unprotected sugar. In the extreme, the protecting group may simply depart with the negative charge during fragmentation and, in so doing, eliminate the possibility of obtaining any information about fragmentation of the carbohydrate portion of the ion. Even with these possible "drawbacks", we considered the potential value of this approach to be great enough to justify testing it by studying several, simple glycosides.

The compounds selected for study were the α and β anomers of methyl 3-*O*-benzyl-2,6-dideoxy-D-*arabino*-hexopyranosides (**1** and **2**). These partially protected sugars were

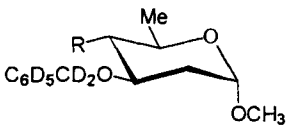
chosen because they have the basic hexopyranoside structure but with only one hydroxyl group available for deprotonation.[23] Benzyl ether protection was chosen because the benzyloxy anion is less likely than other anions, derived from common protecting groups (e.g., benzoate, acetate), to depart from the deprotonated sugar carrying the negative charge. Our goal was to establish the basic fragmentation patterns for the negative ions **3** and **4** and, in future work, turn our attention to the study of regio- and stereoisomers of **3** and **4** as well as the unprotected sugars themselves.



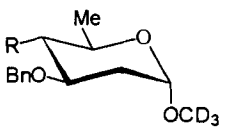
1 R = OH
3 R = $O^{\ominus}$



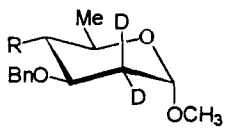
2 R = OH
4 R = $O^{\ominus}$



5 R = OH
6 R = $O^{\ominus}$



7 R = OH
8 R = $O^{\ominus}$



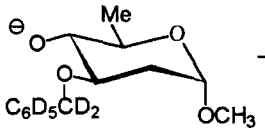
9 R = OH
10 R = $O^{\ominus}$

RESULTS AND DISCUSSION

When methyl 3-*O*-benzyl-2,6-dideoxy-α-D-*arabino*-hexopyranoside (**1**) was deprotonated under negative-ion, chemical-ionization conditions with ammonia as the reagent gas, the corresponding $[M-H]^-$ ion (**3**) was formed along with the cluster ion $[2M-H]^-$. Collisionally activated dissociation (CAD) of **3** produced daughter ions with *m/z* values (and relative abundances) of 219 (3.2), 143 (9.6), 111 (82.3), 107 (2.8), 83 (7.1) and 81 (2.8). These observed mass to charge ratios corresponded to ions that tentatively could be assigned the formulas: $[M-H-CH_3OH]^-$ (*m/z* 219), $[M-H-C_6H_5CH_2OH]^-$ (*m/z* 143), $[M-H-C_6H_5CH_2OH-CH_3OH]^-$ (*m/z* 111), $[C_6H_5CH_2O]^-$ (*m/z* 107), and $[M-H-C_6H_5CH_2OH-CH_3OH-CO]^-$ (*m/z* 83). To understand how these ions formed, we gathered information about reactions of their deuterated analogs.

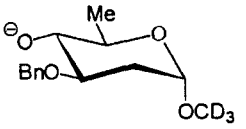
The ion most easily identified was that with *m/z* 107. By comparing the ions produced from **3** with those generated from its partially deuterated analog **6**, the fragment ion with *m/z*

107 (from **3**) was determined to be the benzyloxy anion (eq 1). This determination was possible because CAD of **6** produced a new ion with *m/z* of 114 at the expense of the ion with *m/z* of 107 (Table 1).



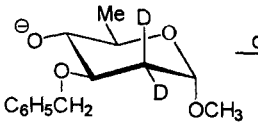
6

CAD → $C_6D_5CD_2OH$ + $C_6D_5CD_2O^{\ominus}$ (*m/z* = 114) + unknown ion (*m/z* = 143) (1)



8

CAD → CD_3OH + unknown ion (*m/z* = 219) (2)



10

CAD → CH_3OD + unknown ion (*m/z* = 220)

CAD → $C_6H_5CH_2OD$ + unknown ion (*m/z* = 144) (3)

Among the other ions formed from CAD of **3**, that with *m/z* 219 corresponded to loss of methanol and that with *m/z* 143 to a loss of benzyl alcohol. These proposed molecular losses were confirmed by study of the deuterium labeled ions **6** and **8**. Elimination of CD_3OH by CAD of **8** gave an ion with *m/z* 219 (eq 2), and CAD of **6** formed the *m/z* 143 ion by elimination of $C_6D_5CD_2OH$ (eq 1). The loss of each of these neutral molecules required, in addition to the departure of a deuterated substituent group, the removal of a proton from the carbon chain of the hexopyranoside ring. Since protons on carbon atoms adjacent to those bearing the deuterated benzyloxy and methoxy groups were likely candidates, we investigated the 2,2'-dideuterio ion **10**. CAD of **10** caused departure of CH_3OD to give an ion with *m/z* 220 and loss of $C_6H_5CH_2OD$ to produce an ion with *m/z* 144 (eq 3). By forming these ions, fragmentation of **10** identified C-2 as the source of the protons lost from **3** when methanol

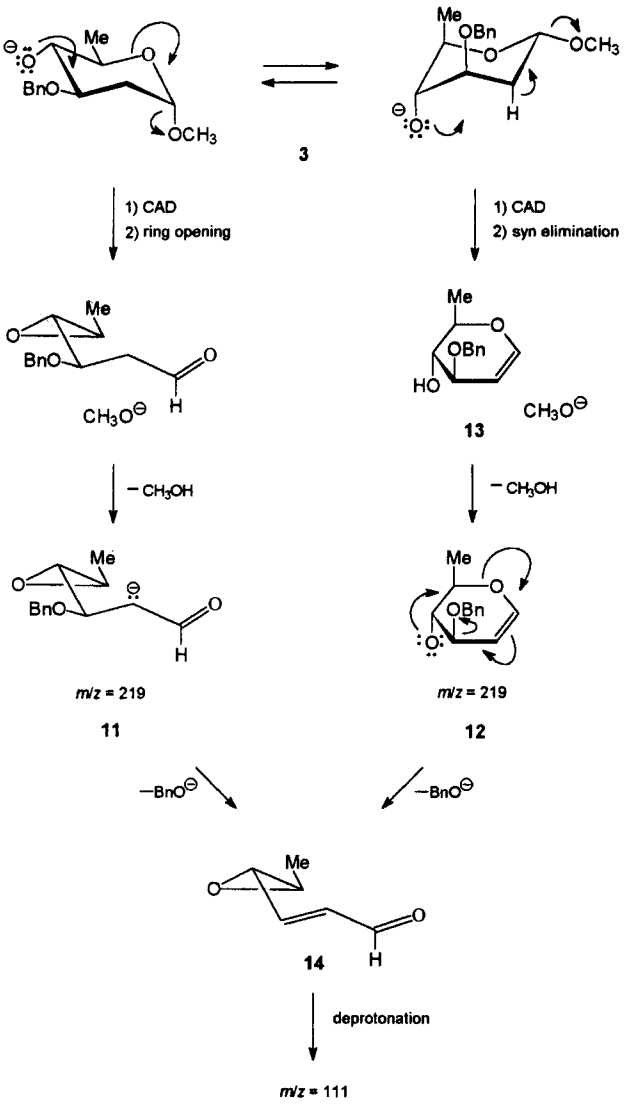
Table 1. Collisionally Activated Dissociation (CAD) of Ions **3, 4, 6, 8, 10, 12, 15, 16, 18, 20, 22, 24**[a]

m/z	**3**	**4**	**6**	**8**	**10**	**12**[b]	**15**	**16**[c]	**18**	**20**	**22**	**24**	m/z
226	-----	-----	2.10	-----	-----	-----	-----	-----	-----	-----	-----	-----	226
220	-----	-----	-----	-----	1.28	-----	2.51	-----	1.11	2.54	1.67	2.22	220
219	3.24	1.18	-----	1.00	-----	-----	2.11	-----	-----	-----	-----	-----	219
146	-----	-----	-----	2.74	-----	-----	-----	-----	-----	-----	-----	-----	146
144	----	-----	-----	-----	1.07	-----	11.2	-----	4.39	7.65	4.70	4.06	144
143	9.56	6.86	8.17	-----	-----	-----	-----	-----	-----	-----	-----	-----	143
114	----	-----	3.91	-----	-----	-----	-----	-----	-----	-----	-----	-----	114
113	----	-----	-----	-----	7.82	-----	-----	-----	-----	-----	-----	-----	113
112	----	-----	-----	-----	15.2	-----	69.0	-----	26.4	64.0	7.22	26.3	112
111	82.3	41.8	57.8	23.0	-----	80.8	32.5	25.0	-----	-----	29.4	2.67	111
110	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	110
107	2.81	1.51	-----	2.73	0.84	3.05	3.60	-----	1.28	2.10	1.32	0.88	107
105	----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	105
84	----	-----	-----	-----	2.31	-----	3.62	-----	1.90	4.72	0.98	1.76	84
83	7.12	4.22	10.4	1.29	-----	13.6	3.87	14.7	-----	-----	1.61	-----	83
82	----	-----	-----	-----	1.11	-----	1.21	-----	-----	1.72	0.78	0.66	82
81	2.76	1.71	4.16	0.96	-----	4.42	2.13	6.13	1.27	-----	0.82	0.64	81

a. The pseudomolecular ion $[M-H]^-$ has a relative abundance of 100 for each ion.
b. The pseudomolecular ion $[M-H]^-$ (*m/z* 219) was subjected to CAD.
c. The pseudomolecular ion $[M-H]^-$ (*m/z* 143) was subjected to CAD.

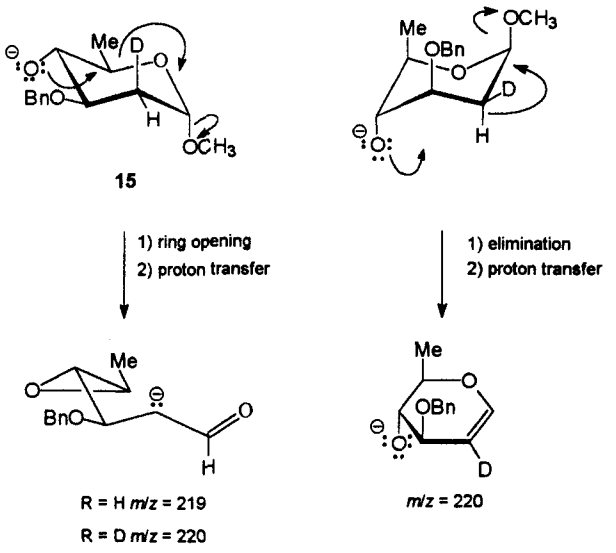
and benzyl alcohol departed.[24] These results eliminated a number of potential *m/z* 219 and 143 ions (eq 1 and 2) and allowed us to focus attention on the most probable remaining structures for these ions.

Two possible structures (**11** and **12**) for the *m/z* 219 ion are shown in Scheme 1 along with proposed mechanisms for their formation. Both **11** and **12** satisfy the requirements established by the deuterium labeling experiments. A difference between their mechanisms



Scheme 1

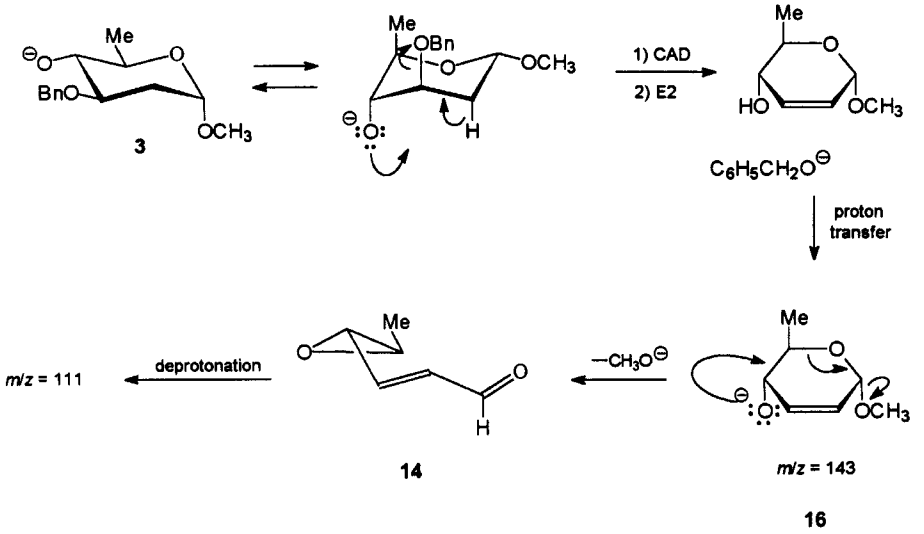
of formation does exist, however, in the identity of the proton that must depart from C-2 during reaction. In the formation of ion **12** (Scheme 1) only the proton on the α-face of **3** can be transferred to O-4 as a part of the syn elimination of the elements of methanol. Formation of the ion **11** involves ring opening prior to proton transfer; therefore, either of the C-2 protons could be abstracted in this process. CAD of the monodeuterio ion **15** produced



Scheme 2

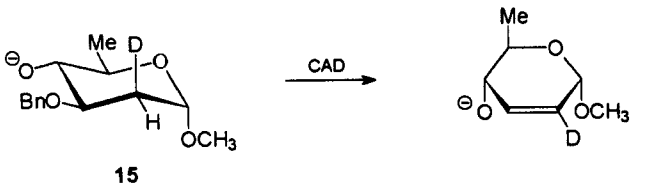
approximately equal amounts of ions with *m/z* 219 (2.11) and 220 (2.51), a result that favored the ring-opening pathway (Scheme 2). Did this result exclude **12** as a reaction intermediate? We do not believe it did. Even though **12** was eliminated as a candidate for the *m/z* 219 ion, it still may be an intermediate in the formation of the *m/z* 111 ion if **12** fragments so quickly after formation that only its fragment ion or ions are observed. If this is the case, CAD of **12** would produce some or even all of the same ions (other that *m/z* 143) observed from CAD of **3**. Collisionally activated dissociation of **12**, formed by deprotonation of the glycal **13**, produced daughter ions with *m/z* (RA) of 111 (80.84), 107 (3.05), 83 (13.61), and 81 (4.42); consequently, the deprotonated glycal **12** remains a possible intermediate in the formation of the *m/z* 111 ion from **3**.

The experiments conducted in determining the structure of ion **11** (*m/z* 219) also were valuable in deciding upon the most probable structure for the *m/z* 143 ion. CAD of ions **6** and **10** (eq 2 and 3) had established that formation of the *m/z* 143 ion involved loss of the benzyloxy anion from C-3 and a proton from C-2. This information suggested the structure **16** for the *m/z* 143 ion and supported the E2 elimination mechanism shown in Scheme 3. For this reaction to take place, the C-2 proton lost would have to come from the α-face of **3**.



Scheme 3

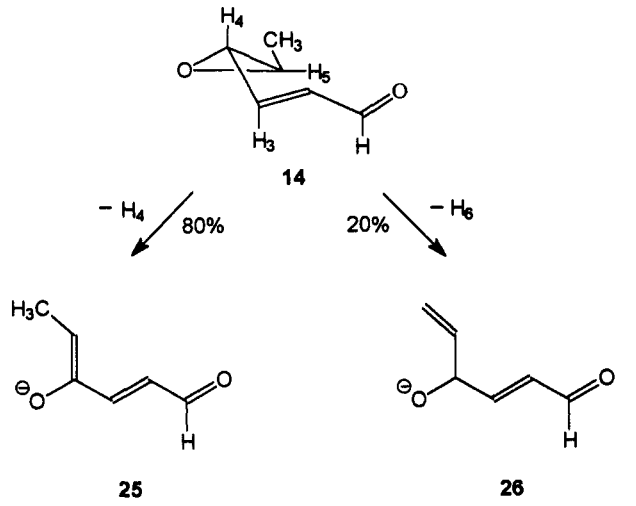
CAD of the monodeuterio ion **15** supported this mechanism (Scheme 3) by demonstrating that only the C-2 proton on the α-face of **15** was lost during reaction (eq 4).



(4)

Schemes 1 and 3 contain steps linking ions **11** (*m/z* 219) and **16** (*m/z* 143) to the major daughter ion (*m/z* 111) from fragmentation of **3**. The element not included in these schemes is a proposed structure for this ion (m/z *111*) because such information requires a determination of the point of deprotonation of **14** (Schemes 1 and 3). Deuterium labeling experiments were essential in establishing this position of proton loss. CAD of the deuterium labeled ions **10**, **18**, and **20** produced no *m/z* 111 ion (Table 1); thus, deprotonation of **14** did not occur from C-1, C-2, or C-3.

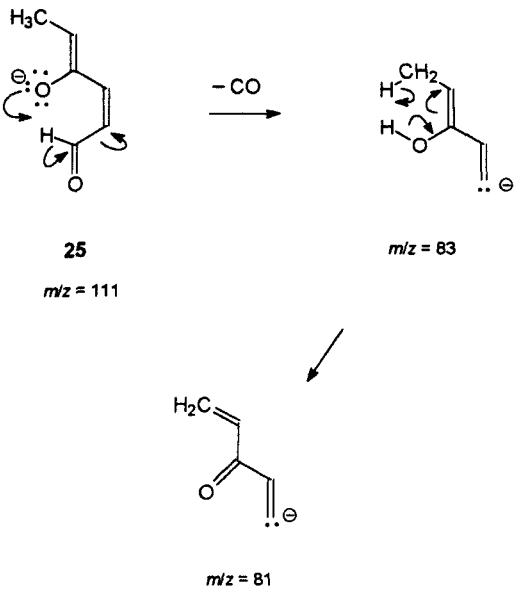
CAD of the 4-deuterio ion **22**, however, did show substantial loss of the deuterium at C-4 during formation of the *m/z* 111 ion. Judging from the relative abundances of the ions



Scheme 4

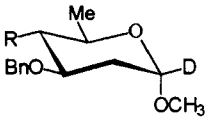
with *m/z* 112 (RA 7.22) and 111 (RA 29.4) from CAD of **22**, one can estimate that about 80% of the proton loss from **14** occurred from C-4 (Scheme 4). This result means that the *m/z* 111 ion from fragmentation of **3** actually is a mixture of ions for which the proposed structure for the major component is **25** (Scheme 4). The other proton lost in forming the *m/z* 111 ions is attached to C-6. CAD of the 6-deuterio isomer **24** showed sufficient deuterium loss to account for the remaining 20% of the deprotonation of **14** not arising from loss of H-4 (Scheme 4).

The final two ions in this fragmentation sequence are those with *m/z* 83 and 81. The *m/z* 83 ion can form from **25** (*m/z* 111) by loss of carbon monoxide. A proposal for how this reaction occurs is shown in Scheme 5. If one assumes that the *m/z* 83 ion is itself a precursor to that with *m/z* 81, then loss of the elements of H_2 must be taking place. What is the source of the hydrogen atoms in H_2? CAD of the 1-deuterio ion **18** shows that deuterium is retained in the sequence of ions until *m/z* 81 ion is reached; therefore, one of the hydrogen atoms that is lost in conversion of the *m/z* 83 into *m/z* 81 ion is H-1. CAD of **24** indicates that the other hydrogen atom making up the departing H_2 originally was attached to C-6 in **3**. A proposal for how this reaction could occur is found in Scheme 5.



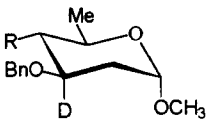
Scheme 5

Once reasonable pathways had been established for fragmentation reactions of **3**, we turned to investigation of its β-anomer (**4**) and found that **4** fragmented to produce exactly the same ions as did **3**. Although it is possible that these two ions (**3** and **4**) formed the same fragment ions by different mechanisms, we believe that this is unlikely; therefore, we concluded that an identical set of deuterium labeling experiments for **4** was not justified. This lack of sensitivity in fragmentation to anomeric configuration increased our interest in investigating structural isomers of **3** and **4** to determine if anomeric stereochemistry is influential in promoting different fragmentation when the deprotonated hydroxyl group is at a different position on the carbon chain. The present results, however, do establish that stereochemistry is an important factor in some of the fragmentation reactions of **3** (e.g., loss of benzyl alcohol, Scheme 3). Also, although the benzyl protecting group definitely is involved in some observed reactions, it does not obscure the ring-opening process (Scheme 1), a reaction that appears likely to characterize unprotected glycosides.



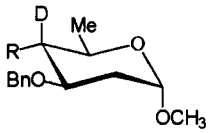
17 R = OH

18 R = $O^{\ominus}$



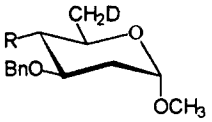
19 R = OH

20 R = $O^{\ominus}$



21 R = OH

22 R = $O^{\ominus}$



23 R = OH

24 R = $O^{\ominus}$

EXPERIMENTAL

General Procedures. Mass spectra were obtained with a Finnigan TSQ-45 triple quadrupole mass spectrometer under the following conditions: source temperature, 120 ^{0}C; ammonia gas pressure, 0.35 Torr; electron energy 70 eV. The collision cell pressure was 1.3 mTorr and the collision energy was 1.2 eV. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were determined in $CDCl_3$. Column chromatography was conducted using a 2.5 x 15 cm column of 240-400 mesh silica gel with hexane–ethyl acetate (3:1) as the developer. TLC was done using silica gel plates developed with ethyl acetate–hexane (1:9), unless otherwise noted.

Methyl 3-*O*-Benzyl-2,6-dideoxy-α-D-*arabino*-hexopyranoside (1) and Methyl 3-*O*-(Benzyl-d_7)-α-D-*arabino*-hexopryanoside (5). Compound **1** was synthesized according to the procedure of Monneret et al.[25] and had the same physical properties reported for this compound.[25,26] The NMR spectra for **1** are included here because they will be referred to in describing its deuterated analogs. ^{1}H NMR ($CDCl_3$): δ 1.30 (H_6, $J_{5,6}$ = 6.2 Hz), 1.62 (H_{2a}, $J_{1,2a}$ = 3.6 Hz, $J_{2a,3}$ = 11.5 Hz, $J_{2a,2e}$ = 12.9 Hz), 2.27 (H_{2e}, $J_{1,2e}$ = 1.3 Hz, $J_{2e,3}$ = 4.9 Hz), 3.22 (H_4, $J_{3,4}$ = 9.1 Hz, $J_{4,5}$ = 9.3 Hz), 3.26 (CH_3O), 3.64 (H_5), 3.73 (H_3), 4.76 (H_1), 4.65 and 4.80 (CH_2, J_{CH2} = 11.6 Hz), 7.33-8.10 (aromatic). ^{13}C NMR: δ 17.88, (C_6), 34.76 (C_2), 54.43 (CH_3O), 67.39 (C_5), 71.10 (CH_2), 76.10 (C_4), 77.09 (C_3), 98.36 (C_1), 127.74, 128.40, 130.08, 133.42 (aromatic).

The synthesis of compound **5** was identical to that of **1** except that $C_6D_5CD_2Br$ was used in place of $C_6H_5CH_2Br$. The physical properties of **1** and **5** were the same except for the lack of aromatic proton resonances in the ^1H NMR spectrum of **5** and aromatic carbon resonances for **5** too weak to be observed in the ^{13}C NMR spectrum.

Methyl 3-*O*-Benzyl-2,6-dideoxy-ß-D-*arabino*-hexopyranoside (2). Methyl 2,6-dideoxy-ß-D-*arabino*-hexopyranoside[27] (573 mg, 3.54 mmol) and 917 mg (3.54 mmol) of tetrabutylammonium hydroxide were dissolved in 15 mL of THF. Benzyl bromide (605 mg, 3.53 mmol) was added and the reaction mixture was stirred for three h. The solution was then filtered through a 2 cm column of silica gel and, after the solvent was evaporated under reduced pressure, the residue chromatographed on a 2.5 x 10 cm column of silica gel to yield 130 mg (0.52 mmol, 15%) of **2**, R_f = O.20 (1:4, ethyl acetate–hexane). ^1H NMR ($CDCl_3$): δ 1.34 (H_6, $J_{5,6}$ = 5.9 Hz), 1.54 (H_{2a}, $J_{1,2a}$ = 9.8 Hz, $J_{2a,3}$ = 11.4 Hz, $J_{2a,2e}$ = 12.6 Hz), 2.32 (H_{2e}, $J_{1,2e}$ = 2.0 Hz, $J_{2e,3}$ = 4.8 Hz), 3.20 (H_4, $J_{3,4}$ = 8.5 Hz, $J_{4,5}$ = 9.0 Hz), 3.48 (CH_3O), 3.30 (H_5), 3.40 (H_3), 4.35 (H_1), 4.47 and 4.68 (CH_2, J_{CH2} = 11.6 Hz), 7.33-8.10 (aromatic). ^{13}C NMR: δ 17.88, (C_6), 35.89 (C_2), 56.51 (CH_3O), 71.60 (C_5), 70.86 (CH_2), 75.72 (C_4), 78.88 (C_3), 100.64 (C_1), 127.88, 127.95, 128.58, 138.00 (aromatic).

Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.64; H, 7.99. Found: C, 66.33; H, 7.75.

Also formed was 170 mg (0.67 mmol, 19%) of methyl 4-*O*-benzyl-2,6-dideoxy-ß-D-*arabino*-hexopyranoside, R_f = 0.18 (1:4, ethyl acetate–hexane). ^1H NMR ($CDCl_3$): δ 1.39 (H_6, $J_{5,6}$ = 6.2 Hz), 1.61 (H_{2a}, $J_{1,2a}$ = 9.6 Hz, $J_{2a,3}$ = 11.9 Hz, $J_{2a,2e}$ = 12.5 Hz), 2.20 (H_{2e}, $J_{1,2e}$ = 2.0 Hz, $J_{2e,3}$ = 5.1 Hz), 3.00 (H_4, $J_{3,4}$ = 8.9 Hz, $J_{4,5}$ = 8.9 Hz), 3.49 (CH_3O), 3.35 (H_5), 3.71 (H_3), 4.39 (H_1), 4.78 and 4.70 (CH_2, J_{CH2} = 12.0 Hz), 7.36-8.10 (aromatic). ^{13}C NMR: δ 18.30, (C_6), 38.70 (C_2), 56.53 (CH_3O), 71.14 (C_5), 75.18 (CH_2), 85.96 (C_4), 71.21 (C_3), 100.56 (C_1), 127.94, 128.11, 128.70, 133.42 (aromatic).

Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.64; H, 7.99. Found: C, 66.45; H, 8.11.

Methyl-d_3 3-*O*-Benzyl-2,6-dideoxy-α-D-*arabino*-hexopyranoside (7). Methyl 3-*O*-benzyl-2,6-dideoxy-ß-D-*arabino*-hexopyranoside (**1**, 50 mg, 0.20 mmol) was dissolved in a 2% solution of DCl in 2.0 mL of CD_3OD. After stirring for 2 h, the solvent was evaporated under a stream of nitrogen and the residue chromatographed on a 2.5 x 10 cm column of silica gel to yield 30 mg (0.12 mmol, 60%) of 7. This material was identical in physical

properties to **1** except that resonances for the methoxy protons were absent in ^{1}H NMR spectrum and the resonance for the carbon atom in the methoxy group was too weak to be observed in the ^{13}C NMR spectrum.

Methyl 3-*O*-Benzyl-2,6-dideoxy-2,2'-dideuterio-ß-D-*arabino*-hexopyranoside (9). 4-*O*-Benzoyl-3-*O*-benzyl-2,6-dideoxy-D-*arabino*-hexopyranose[28] (200 mg, 0.58 mmol) was dissolved in 4 mL of CD_3OD to which about 20 mg of sodium had been added. The reaction mixture was allowed to stir overnight under nitrogen and then acidified with a 5% solution of DCl in CD_3OD. The reaction mixture was heated under reflux for one h and then the solvent was removed by evaporation under reduced pressure at room temperature. The residue was chromatographed in the standard fashion to yield 100 mg (0.39 mmol, 68%) of **9**. This material was identical in ^{1}H NMR spectra to **1** except that the resonances for H_2 and $H_{2'}$ were not present and there was no coupling between H_2 and H_1 or H_3. The resonance for C_2 in the ^{13}C NMR spectrum was too weak to be observed.

1,5-Anhydro-3-*O*-benzyl-2,6-dideoxy-D-*arabino*-hex-1-enitol (13). The glycal **13** was synthesized according to the procedure of Thiem, Klaffke, and Springer.[29]

Methyl 3-*O*-Benzyl-2$_a$-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside. Methyl 4,6-*O*-benzylidene-2$_a$-deuterio-2-deoxy-α-D-*ribo*-hexopyranoside (550 mg, 3.40 mmol), synthesized according to the procedure of Baer and Hanna,[30] 621 mg (3.49 mmol) of *N*-bromosuccinimide, and 1.18 g (6.0 mmol) of barium carbonate were combined with 50 mL of benzene and heated under reflux for 30 min. The reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was extracted with 3 x 100 mL of hexane, and the solvent was removed from the filtrate and the combined hexane extracts. This material was dissolved in 20 mL of methylene chloride containing 1.3 mL of pyridine. The solution was cooled to -20 °C and 1.2 mL of triflic anhydride in 3 mL of methylene chloride was added in a dropwise fashion. After allowing the reaction mixture to rise to room temperature over a period of 1 h, the solvent was removed under reduced pressure. The residue was extracted with 3 x 50 mL of boiling hexane, and the solvent was then removed from the hexane extracts under reduced pressure to give the triflate. Excess pyridine was removed by three coevaporations with toluene under reduced pressure. The triflate was immediately combined with 1 mL of benzyl alcohol and 460 mg (2.27 mmol) of 4-methyl-2,6-di-*tert*-butyl-

pyridine and heated for three h at 110 °C. Chromatography of the reaction mixture produced a clear oil that was treated with 5 mL of a 1.0 M solution of lithium triethylborohydride in tetrahydrofuran and stirred for 4 h. The reaction mixture was treated with 5 mL of 15% hydrogen peroxide (exothermic reaction), and the solvent was removed under reduced pressure. The residue was chromatographed in the standard fashion to give 153 mg (0.60 mmol, 19%) of methyl 3-*O*-benzyl-2_a-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside. This material had a ^{1}H NMR spectrum identical to that of compound **1** except that the H_{2a} resonance was missing and the coupling between H_{2a} and other protons no longer could be observed. The proton decoupled ^{13}C NMR spectrum also was identical to that of **1** except that the resonance at δ 34.81 (C-2) was split into three peaks (J = 19.7 Hz).

Methyl 3-*O*-Benzyl-1-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside (17). 3-*O*-Benzyl-2,6-dideoxy-D-glucono-1,5-lactone (50 mg, 0.15 mmol) was dissolved in 2.0 mL of methanol and 20 mg of sodium borodeuteride was added. After stirring for 15 min, the solvent was evaporated and the residue was chromatographed to give a clear oil that was combined with 33 mg (0.15 mmol) of pyridinium chlorochromate and dissolved in 5 mL of dichloromethane. After 14 h, the reaction mixture was filtered through a 1 cm pad of silica gel and the silica gel was washed with 5 mL of dichloromethane. The dichloromethane was evaporated from the combined solutions, and the residue was dissolved in 10 mL of a 2% solution of HCl in methanol. This solution was heated under reflux for 2 h. After removal of the solvent, the residue was chromatographed in the standard fashion to give 32 mg (0.10 mmol, 66%) of compound **17**, identical in ^{1}H NMR spectrum to that of **1** except that the resonance for H-1 and the coupling between H_1 and the protons attached to C_2 were not present. The ^{13}C NMR spectrum of **17** also was identical to the spectrum of **1** except that the resonance at 98.31 (C_1) was too weak to be observed.

Methyl 3-*O*-Benzyl-3-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside (19). A solution of methyl 4-*O*-benzoyl-2,6-dideoxy-α-D-*arabino*-hexopyranoside[31] (0.120 g, 0.48 mmol) in toluene (5 mL) was stirred vigorously and heated under reflux while 0.55 g (2.5 mmol) of pyridinium chlorochromate was added. After 1 h, the reaction mixture was filtered through Celite and the filtrate was concentrated and chromatographed in the standard manner to give 0.11 g of crude ketose. This material was dissolved immediately in a mixture of 1.0

mL of ethanol and 0.15 mL of water and the solution was rapidly stirred while 45 mg of sodium borodeuteride was added. The solvent was evaporated from the reaction mixture under reduced pressure and the residue was partitioned between water (1 mL) and 2 mL of dichloromethane. The layers were separated and the aqueous layer was extracted with 2 x 1 mL of dichloromethane. The combined organic extracts were concentrated to a syrup, which was chromatographed according to the standard procedure to give 40 mg (0.16 mmol, 33%) of methyl 4-*O*-benzoyl-3-deuterio-2,6-dideoxy-α-D-*ribo*-hexopyranoside, identical in physical properties to an authentic sample[31] except that the resonance at δ 65.55 (C_3) in the ^{13}C NMR spectrum was too weak to be observed. Also missing were the resonance at δ 4.19 (H_3) and the coupling constants $J_{3,4}$, $J_{2e,3}$, and $J_{2a,3}$ from the ^1H NMR spectrum. Also isolated from chromatography was 30 mg (0.12 mmol, 25%) of methyl 4-*O*-benzoyl-3-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside, identical in physical properties with an authentic sample[31] except that the resonance at δ 66.94 was absent from the ^{13}C NMR spectrum and the resonance at δ 3.70, along with the coupling constants $J_{3,4}$, $J_{2e,3}$, and $J_{2a,3}$ from the ^1H NMR spectrum.

Methyl 4-*O*-benzoyl-3-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside was converted into methyl 3-*O*-benzyl-3-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside (**19**) according to the procedure of Monneret et al.[25] This compound was identical in spectral properties to **1** except that the resonances for C_3 was too weak to be observed and that for H_3 was absence from the NMR spectra.

Methyl 3-*O*-Benzyl-4-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside (21). A solution of methyl 3-*O*-benzyl-2,6-dideoxy-α-D-*arabino*-hexopyranoside (**1**, 0.239 g, 0.95 mmol) in toluene (5 mL) was stirred vigorously and heated under reflux while 0.51 g (2.32 mmol) of pyridinium chlorochromate was added. After 7 h, the reaction mixture was filtered through Celite and the reaction vessel and Celite were washed with an additional 2 mL of toluene. The filtrate was concentrated under reduced pressure and then chromatographed on a 2.5 x 15 cm column of 240-400 mesh silica gel which was eluted with 1:4 ethyl acetate–hexane to give 0.23 g of crude ketose. This compound was dissolved immediately in a mixture of 1.0 mL of ethanol and 0.15 mL of water, and the solution was rapidly stirred while 45 mg of sodium borodeuteride was added. The solvent was evaporated from the

reaction mixture under reduced pressure, and the residue was partitioned between water (3 mL) and ethyl acetate 5 mL. The layers were separated and the aqueous layer was extracted with 2 x 5 mL of ethyl acetate. The combined organic extracts were dried over sodium sulfate and concentrated to a syrup, which was chromatographed according to the standard procedure to give 180 mg (76%, R_f 0.51, 1:1 ethyl acetate–hexane) of methyl 3-*O*-benzyl-4-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside (**21**). This compound had the same physical and spectral properties as **1** except that the ^1H NMR spectrum had no resonance at δ 3.22 and exhibited no $J_{3,4}$ and $J_{4,5}$ coupling constants. The resonance for C_4 in the ^{13}C NMR spectrum was too weak to be observed. A small amount (30 mg) of a second compound which had ^1H NMR and ^{13}C NMR spectra consistent with methyl 3-*O*-benzyl-4-deuterio-2,6-dideoxy-α-D-*lyxo*-hexopyranoside was isolated.

Methyl 3-*O*-Benzyl-6-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside (23). Methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside, synthesized according to the procedure of Baer and Hanna,[30] was treated in exactly the same manner as described in the synthesis of methyl 3-*O*-benzyl-2_a-deuterio-2,6-dideoxy-α-D-*arabino*-hexopyranoside except that the lithium triethylborohydride in the final step was replaced by lithium triethylborodeuteride. The product was identical in ^1H NMR spectrum to compound **1** except that one of the H-6 resonances was missing. The proton decoupled ^{13}C NMR spectrum also was identical to that of **1** except that the resonance at δ 17.89 (C_1) was split into three peaks (J = 20 Hz).

ACKNOWLEDGMENT

The authors thank the Lewis Research Center (NASA) for the donation of the mass spectrometer used in this research.

REFERENCES AND NOTES

1. M. Barber, R. S. Bordoli, R. D. Sedgwick and J. C.Vickerman, *J. Chem. Soc., Faraday Trans.*, **78**, 1291 (1982).
2. R. Guevremont and J. L. C. Wright, *Rapid Commun. Mass Spectrom.*, **1**, 1 (1987).
3. J. W. Dallinga, F. D. Rinkerma and W. Heerma, *Biomed. Environ. Mass Spectrom.*, **18**, 241 (1989).

4. J. W. Dallinga and W. Heerma, *Biomed. Environ. Mass Spectrom.*, **18**, 363 (1989).
5. D. Garozzo, M. Giuffrida, G. Impallomeni, A. Ballistreri and G. Montaudo, *Anal. Chem.*, **62**, 279 (1990).
6. J. W. Dallinga and W. Heerma, *Biol. Mass Spectrom.*, **20**, 215 (1991).
7. J.-C. Prome, H. Aurelle, D. Prome and A. Savagnac, *Org. Mass Spectrom.*, **22**, 6 (1987).
8. S. Dua, R. A. J. O'Hair, J. H. Bowie and R. N. Hayes, *J. Chem. Soc., Perkin Trans. 2*, 1151 (1992).
9. A. Ballistreri, G. Montaudo, D. Garozzo, M. Giuffrida and G. Impallomeni, *Rapid Commun. Mass Spectrom.*, **3**, 302 (1989).
10. T. Ii, S. Okuda, T. Hirano, K. Tsujimoto and M. Ohashi, *Org. Mass. Spectrom.*, **28**, 789 (1993).
11. D. Garozzo, G. Impallomeni, G. Montaudo and E. Spina, *Rapid Commun. Mass. Spectrom.*, **6**, 550 (1992).
12. J. A. Carroll, L. Ngoka, C. G. Beggs and C. B. Lebrilla, *Anal. Chem.*, **65**, 1582 (1993).
13. D. T. Li and G. R. Her, *Anal. Biochem.*, **221**, 250 (1993).
14. T. Ii, M. Kubota, S. Okuda, T. Hirano and M. Ohashi, *Glycoconjugate J.*, **12**, 162 (1995).
15. D. G. Hehemann and B. Winnik, *Org. Mass Spectrom.*, **28**, 729 (1993).
16. M. Coppola, D. Favretto, P. Traldi and G. Resnati, *Org. Mass Spectrom.*, **29**, 553 (1994).
17. H.-J. Yang and Y.-Z. Chen, *J. Carbohydr. Chem.*, **12**, 39 (1993).
18. H.-J. Yang, Y.-Z. Chen, F.-Z. Zhao, H.-Q. Li, J.-J. Zhai and N.-Y. Chen, *Chem. Res. Chinese Univ.*, **8**, 231 (1992).
19. H. Li, H.-J. Yang, S.-N. Chen and Y.-Z. Chen, *Chin. J. Chem.*, **10**, 337 (1992).
20. L. Yan and Y.-W. Fang, *Chin. J. Chem.*, **11**, 53 (1993).
21. W. Heerma, C. Versluis, W. Kulik, R. R. Contreras and J. P. Kamerling, *Biomed. Environ. Mass Spectrom.*, **17**, 257 (1988).
22. This critical fact has been emphasized by Dallinga and Heerma in their extensive investigation of D-aldohexoses.[4]
23. Bowie et al.[8] have shown that in model systems a minor amount of deprotonation occurs from carbon atoms rather than hydroxyl groups. This was a possibility also for compound **1**; however, subsequent study of deuterated analogs did not indicate this to be a significant process.
24. A minor but significant pathway exists in which both benzyl alcohol and methanol are lost from **10** without deuterium loss from C-2.
25. C. Monneret, R. Gagnet, and J.-C. Florent, *J. Carbohydr. Chem.*, **6**, 221 (1987).
26. A. O. Stewart and R. M. Williams, *Carbohydr. Res.*, **135**, 167 (1984).
27. R. W. Binkley, *J. Carbohydr. Chem.*, **13**, 111 (1994).
28. R. W. Binkley and D. J. Koholic, *J. Org. Chem.*, **54**, 3577 (1989).
29. J. Thiem, W. Klaffke and D. Springer, *Carbohydr. Res.*, **174**, 201 (1988).
30. H. H. Baer and H. R. Hanna, *Carbohydr. Res.*, **110**, 19 (1982).
31. R. W. Binkley and M. A. Abdulaziz, *J. Org. Chem.*, **52**, 4713 (1987).