ORGANIC MASS SPECTROMETRY, VOL. 22, 6-12 (1987)

Gas Phase Glycosidic Cleavage of Oxyanions from Alkyl Glycosides

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The oxyanion $[M-H]^-$ from several methylglycosides were generated by fast atom bombardment and their decomposition was studied by mass-analysed ion kinetic energy spectrometry. The main decomposition pathway is the loss of methanol. The hydroxylic hydrogen arises by proton transfer from the hydroxyl groups of the sugar. In the gluco-series, no anomeric effect is found. The absence of either the hydroxyl groups at C-2 or C-6 does not inhibit the glycosidic cleavage. However, the blocking of both the hydroxyl groups at C-4 and C-6, by a benzylidene group or two methyl groups, inhibit completely the glycosidic cleavage. From these results, it is proposed that the glycosidic cleavage occurs after opening the sugar ring by a vicinal attack of an oxyanion at C-6 or C-4 to the C-5 carbon atom. Then, the ionized hemi-acetals fragment into a methanolate anion and a 5,6- or 4,5-anhydrosugar which exchange another proton before their separation into charged and neutral species.

INTRODUCTION

Several authors have shown that glycoconjugates could be studied by negative ion, fast-atom bombardment (FAB) mass spectrometry.[1-7] Thus, the molecular weight of the molecule was determined by the presence of intense $[M-H]^-$ ions (molecular oxyanions) and a sequence determination was possible by the study of fragments ions. Among several cleavage pathways, a very important one is the glycosidic cleavage which leads to sequence ions from the non-reducing end of the molecule. From a chemical point of view, this cleavage appears to be an uncommon reaction since it is well known in solution chemistry that glycosidic linkages are resistant towards a nucleophilic attack by oxyanions.

In a preceding paper,[8] we have shown that the main pathway for the glycosidic cleavage by negative chemical ionization (direct exposure in the presence of chloride ions) was a pyrolytic reaction, followed by the ionization of the fragments. However, we have also demonstrated that the cleavage in the gas phase from the chloride adduct does also occur, but to a minor extent.

In the negative ion, fast atom bombardment mass spectra, generated in the ion source, the fragment ions may also arise from two distinct mechanisms; (i) a pyrolytic cleavage in the condensed phase followed by the ionization of the neutral fragments; (ii) the decomposition of the intact oxyanion of the glyconconjugate in the gas phase.

In this paper, we demonstrate that the glycosidic cleavage of oxyanions from several alkyl glycosides occur in the gas phase. We have also shown that this cleavage does not result from a direct attack on the anomeric carbon atom, but from the decomposition of a hemi-acetalic oxyanion formed by opening of the sugar ring.

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0030-493X/87/010006-07$05.00

RESULTS AND DISCUSSION

Unimolecular decomposition of the molecular oxyanions from methylglycopyranosides

The negative ion FAB mass spectra of methyl-α-D-glucopyranoside, in a glycerol matrix, is presented in Fig. 1. It is mainly characterized by the presence of an intense molecular oxyanion at m/z 193, accompanied by solvatation clusters: $[M+\text{glycerol}-H]^-$ at m/z 285, $[M+2\,\text{glycerol}-H]^-$ at m/z 377 and $[2M-H]^-$ at m/z 387. The only fragment ion which could be easily detected from the background was m/z 101. The fragment ion which could arise from a glycosidic cleavage by the loss of methanol was not distinguishable from the background.

However, fragmentations of metastable ions were clearly seen in the spectra measured in the single focusing mode. Some of them correspond to desolvatation reactions, either by losses of glycerol from m/z 285 and 377, respectively, at $m^* = 130.7$ and $m^* = 215.4$, or by the dissociation of the dimer into the molecular oxyanion at $m^* = 96.2$. An intense metastable ion at $m^* = 134.3$ could be attributed to the reaction $m/z\ 193^- \rightarrow m/z\ 161^- + m/z\ 32$, namely to the glycosidic cleavage of the molecular oxyanion of methylglucopyranosides, by loss of methanol.

To verify this attribution, the mass-analysed ion kinetic energy (MIKE) spectra of m/z 193 was recorded (Fig. 2). As expected, it presented a very intense peak corresponding to the unimolecular glycosidic cleavage reaction, $m/z\ 193^- \rightarrow m/z\ 161^- + m/z\ 32$. The other fragment ions are less intense. They are attributed, respectively, to the loss of water (m/z 175), the simultaneous loss of water and methanol (m/z 143) and the formation of the fragment m/z 101. To avoid any ambiguity with a possible loss of 92 mass units from an isobaric solvated fragment from the glycerol matrix, the

Received 6 March 1986
Accepted 25 May 1986

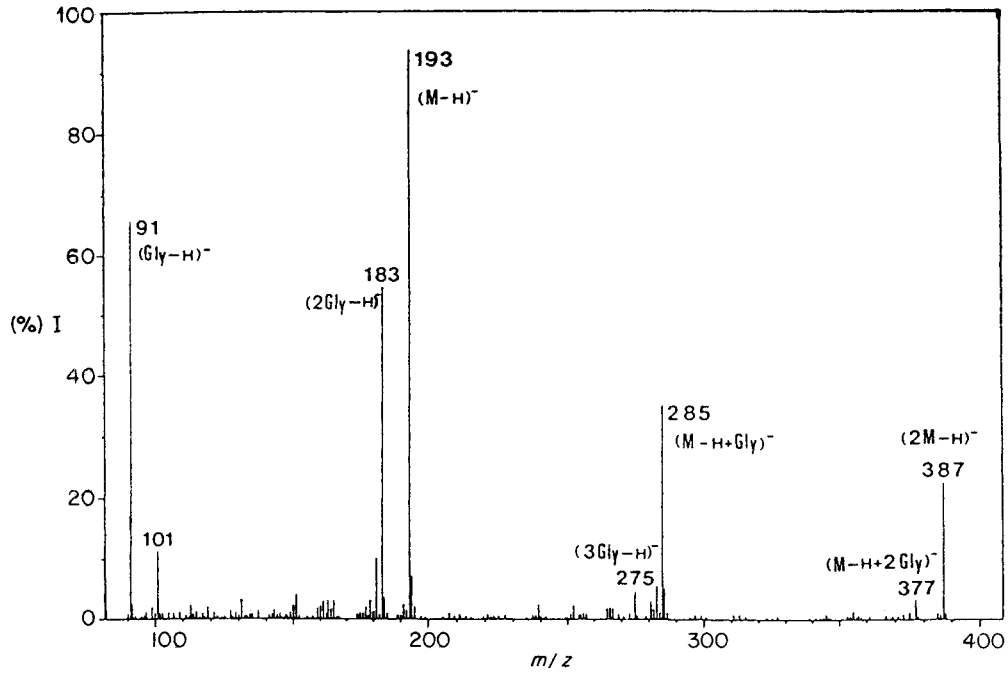


Figure 1. Negative ion FAB mass spectrum of methyl-α-D-glucopyranoside. (Glycerol matrix).

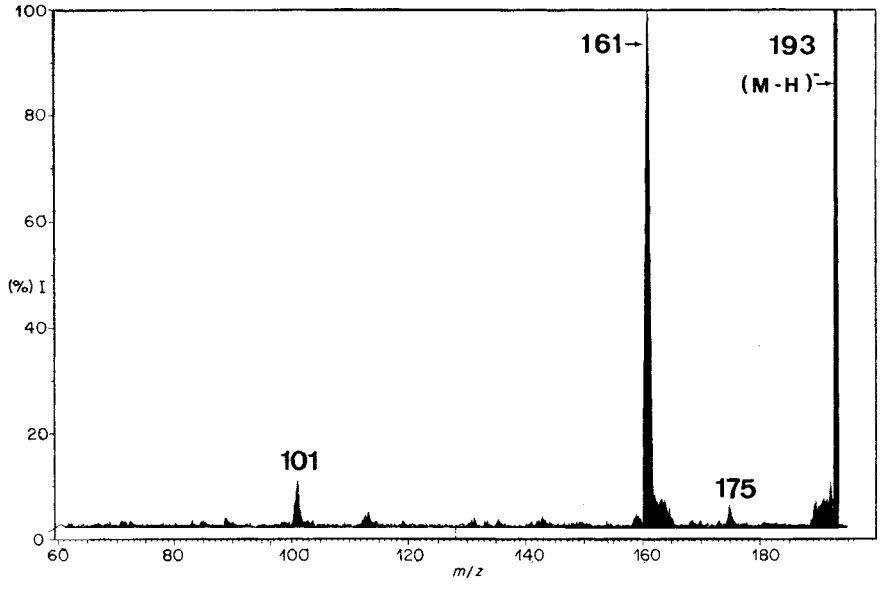


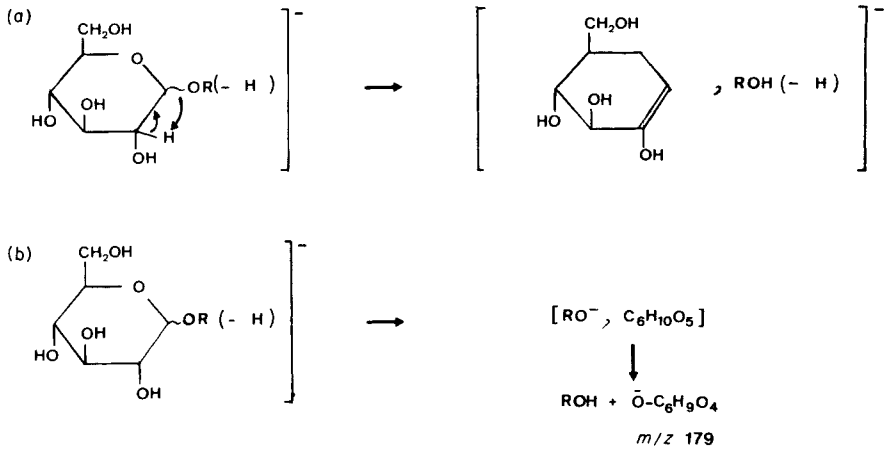
Figure 2. MIKE spectrum of the $[M-H]^-$ ion of methyl-α-D-glucopyranoside (m/z 193).

same experiment was repeated again in diethanolamine. The loss of 92 mass units still remained, showing that this neutral loss arose from the sugar anion.

The main fragment ions in the MIKE spectra of the molecular oxyanion from several methylglycosides are presented in Table 1. It could be seen that the relative abundance of the glycosidic cleavage reaction is not modified by a change in the configuration of either the anomeric carbon atom or the hydroxyl on C-2. However, the abundance of this cleavage is strongly lowered in the galacto-series.

Table 1. Main fragment ions in the MIKE spectra of the $[M-H]^-$ ion from some methylglycopyranosides (the matrix was diethanolamine)

Methylglycopyranoside	Relative abundance (Σ%) $[M-H-H_2O]^-$	$[M-H-CH_3OH]^-$	m/z 101
α-D-gluco-	3.3	89.1	7.5
β-D-gluco-	1.2	88.6	5.7
α-D-manno-	2.4	73.1	14.5
α-D-galacto-	26.0	23.5	13.3
β-D-galacto-	8.3	52.9	18.5



Scheme 1

Origin of the hydroxylic hydrogen in the loss of methanol from the oxyanions of methylglycosides

A proposal for the mechanism of the glycosidic cleavage of oligosaccharides in their negative ion mass spectra has been already presented by others authors.[1-3] According to this scheme, the hydrogen atom transferred to the interglycosidic oxygen atom comes from the C-2—H bond of the sugar ring (Scheme 1(a)). An alternative hypothesis could be a proton exchange between a methanolate anion which has been generated in a first decomposition step, and the remaining part of the sugar. According to this hypothesis, the hydrogen atom in the hydroxylic group of methanol now comes from the most acidic sites of the molecule, i.e. from the hydroxyl groups of the sugar (Scheme 1(b)).

To verify which was the valid assumption, the FAB mass spectra of several methylglycopyranosides were recorded using O-d_3-glycerol in deuterated water as a matrix to exchange active hydrogens by deuterium.[9] In methylglycosides, this method exchanges all hydroxylic protons, and, as expected, the molecular oxyanion was shifted by 3 mass units. The MIKE spectrum of this anion indicated the only loss of CH_3OD. Thus, the hydrogen atom arises specifically from one of the hydroxyl groups of the sugar, according to our hypothesis depicted in Scheme 1(b).

Glycosidic cleavage of oxyanions from modified methylglycosides

Table 2 presents the relative intensity of the fragment ions formed from several modified methylglycopyranosides oxyanions, as measured by their MIKE spectra.

The selective trideuteriomethylation of the hydroxyl group at C-2 in the methyl-α-D-glucopyranoside has only a small influence on the loss of methanol. This result rules out definitively any important role of an oxyanion C-2 for promoting the glycosidic cleavage.

The relative rate of the glycosidic cleavage was decreased but not suppressed by removing the hydroxyl C-6 group. However, the simultaneous protection of both the hydroxyl C-4 and C-6 by a benzylidene group induced a complete suppression of the glycosidic cleavage.

Since this result could be interpretated either as a conformational effect due to the bulky 4-6-linked benzy-

Table 2. Main fragment ions in the MIKE spectra of the $[M-H]^-$ ion from modified glycopyranosides (the matrix was glycerol, the relative abundance of the ions in brackets).

Compound number	Glycopyranoside	*m/z* value and relative abundance of the fragments: Glycosidic cleavage	Other main ions
1	methyl-6-deoxy-α-D-glucopyranoside	*m/z* 145 (42.6)	*m/z* 101 (8.4), *m/z* 85 (18.3) *m/z* 143 (3.3)
2	methyl-6-deoxy-β-D-glucopyranoside	*m/z* 145 (36.4)	*m/z* 101 (5.0), *m/z* 85 (10.6) *m/z* 143 (22.7)
3	methyl-2-*O*-trideuteriomethyl-α-D-glucopyranoside	*m/z* 178 (80.7)	*m/z* 160 (6.3), *m/z* 101 (2.6)
4	methyl-4,6-*O*-benzylidene-α-D-glucopyranoside	*m/z* 249 (<1)	*m/z* 85 (100)
5	methyl-4,6-di-*O*-trideuteriomethyl-β-D-glucopyranoside	*m/z* 195 (<1)	*m/z* 192 (48.6) loss of CD_3OH *m/z* 183 (5.6) *m/z* 160 (19.4), *m/z* 135 (4.5)
6	trideuteriomethyl-4, 6-di-*O*-methyl-D-glucopyranoside (mixture of α and β anomers)	*m/z* 189 (<1)	*m/z* 192 (84.2) loss of CH_3OH *m/z* 157 (6.6)

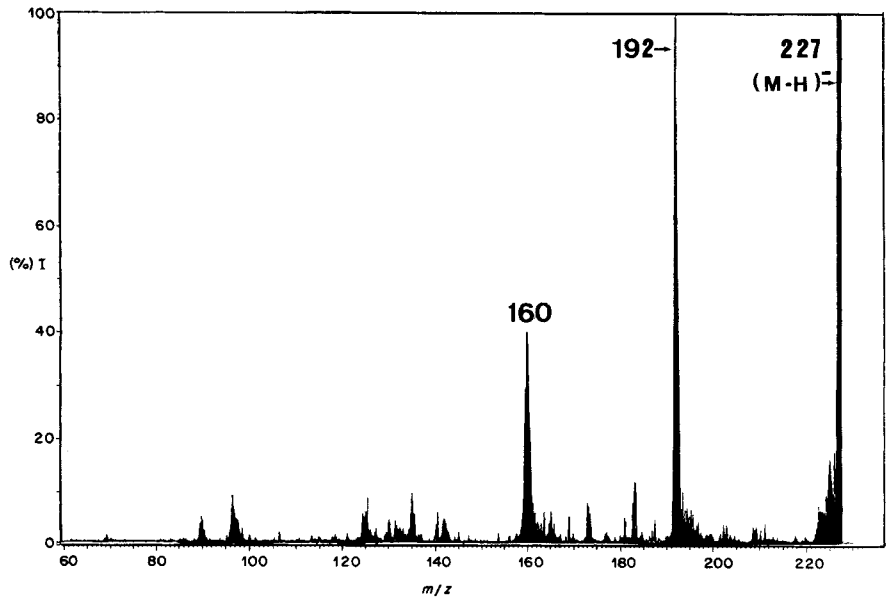


Figure 3 MIKE spectrum of the $[M-H]^-$ ion of methyl-4,6-di-*O*-trideuteriomethyl-β-D-glucopyranoside (*m/z* 227). The base peak corresponded to the loss of trideuterated methanol.

lidene group, or as an indication of the direct role of free C-4 and C-6 hydroxyl groups, the synthesis of 4,6-di-*O*-methyl-analogues was done. These analogues were deuterated either at the 4,6-di-O-methyl groups (Compound **5**) or at the methyl group on C-1 (Compound **6**), in order to avoid any ambiguity on the origin of the methanol losses.

The MIKE spectra of the molecular oxyanion of these compounds clearly shows the absence of any glycosidic cleavage. Compound **5** shows the loss of 35 mass units (CD_3OH) and no loss of 32 mass units (CH_3OH) (Fig. 3). For compound **6**, the loss of CH_3OH is only detected. Thus, this experiment clearly shows that the substitution of both the hydroxyl groups at C-4 and C-6 inhibited the glycosidic cleavage in the molecular oxyanion of methylglycopyranosides.

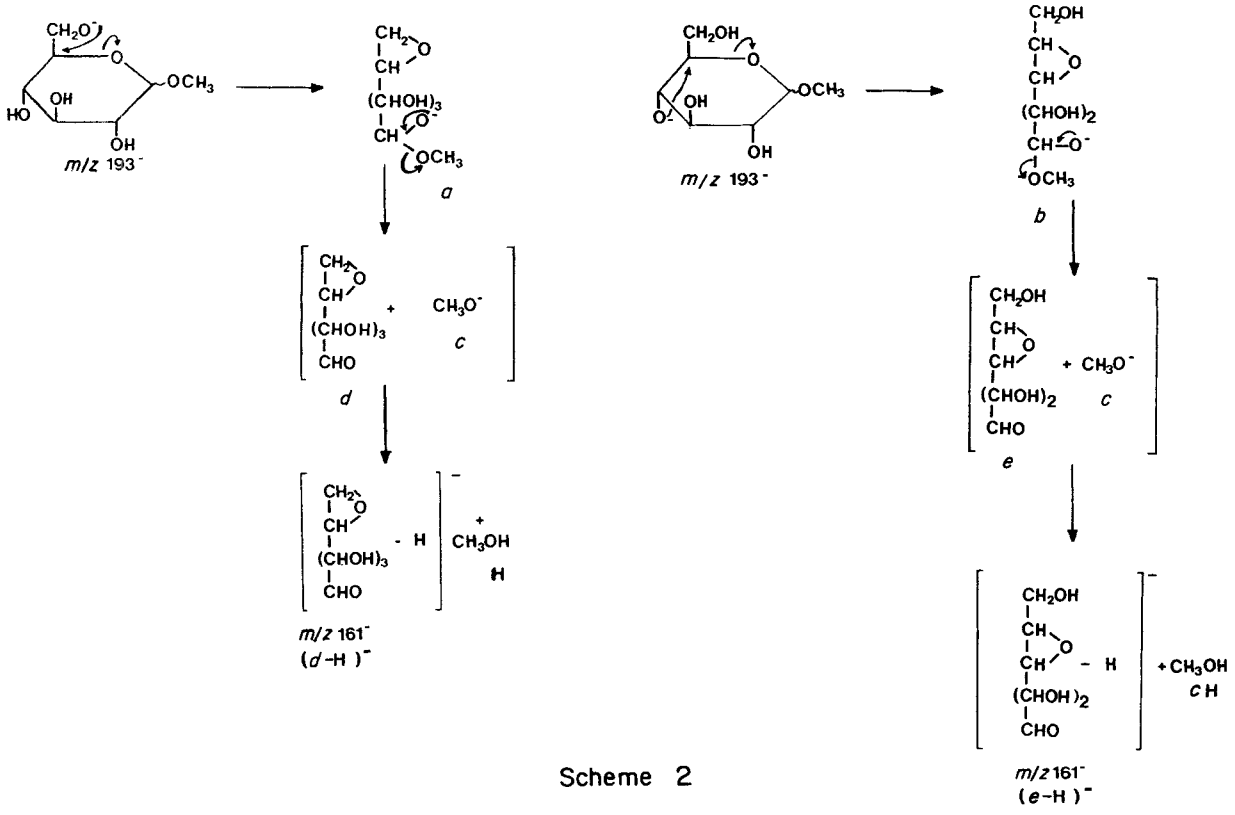
Discussion

In solution chemistry the mechanism for the base-promoted glycosidic cleavage of arylglycosides had been the subject of several papers whereas the cleavage of alkylglycosides was poorly studied because of the need for highly drastic conditions which led to an extensive decomposition of the molecules.[11] Thus, it had been shown that arylglycosides were cleaved by strong bases, such as OH^- or CH_3O^-, through a vicinal attack by the C-2-oxyanion on the anomeric carbon atom.[10] It had been also demonstrated that the reaction rate was much higher when the hydroxyl group at C-2 and the aryloxy-group at C-1 were *trans* relative to each other. Moreover, when the leaving group had the α-configuration, the hydroxyl group at C-6 could participate by formation of 1,6-anhydride.[10]

The mechanism of glycosidic cleavage of methylglycopyranosides oxyanions in the gas phase appears to be different. The hydroxyl group at C-2 does not participate, since the glycosidic cleavage of methylglucopyranosides is not much affected by a 2-O-methylation. Other ionized hydroxyl groups might participate to the cleavage through an intramolecular attack on C-1 by either an oxyanion or a hydride ion leading to a nucleophilic substitution reaction. However, such a reaction needs an antiplanar position of both attacking and leaving groups, and a high stereospecificity at C-1 would be expected. Since the configuration of the anomeric carbon has no effect upon the relative rate for the loss of methanol in the gluco-series, such a hypothesis seems unlikely. Moreover, the substitution of the hydroxyl group at C-6 which is the best position for an intervention with C-1 in a boat conformation does not inhibit the reaction.

Our observation that the gas phase glycosidic cleavage needs a free hydroxyl group either at C-6 or at C-4 leads us to propose the following reaction pathway (Scheme 2). The first step is the opening of the sugar ring by cleavage of the C-5—O bond. This bond is cleaved by the attack on C-5 by a vicinal oxyanion leading to the formation of an epoxide ring either on C-5-C-6, if the C-6-oxyanion was first formed, or alternatively to an epoxide ring on C-5-C-4, if the reaction was initiated by a C-4-oxyanion. These reactions can be considered as the reversal of the action of an alcoholate ion on an epoxide ring. Such a ring opening generates the acyclic deprotonated hemiacetal *a* or *b*, which are cleaved further into the alcoholate anion *c* and aldehydes *d* or *e*. Then, a proton transfer reaction occurs between the neutral (*d* or *c*) and ionized (*c*) fragments, generating a neutral alcohol *c*H and deprotonated anhydrohexoses, $[d-H]^-$ and $[e-H]^-$.

In these schemes, the cleavage of the acetal group occurs from an open form, thus explaining the absence of any anomeric effect observed in the gluco-series. In the galacto-series, the ring opening by the vicinal attack of O-4 would be more difficult since this hydroxyl group possesses an axial position in the more stable chair conformation. This explains the lesser abundance for the loss of methanol in this series.



Scheme 2

However an anomeric effect is clearly seen in the galacto-series (Fig. 4(a) and 4(b)), which can be interpreted as the result of a direct attack of the axial O-4 on C-1 in a boat conformation. For such a pathway, an easier reaction could be expected for the α-anomer. The experimental results show the opposite effect. This apparent anomeric effect is probably due to an increase of the speed of the competitive losses of water and hydrogen from $[M-H]^-$: since the relative abundances of the ions in the MIKE spectra reflect the relative rate of competitive reactions, an increase of some of them induces apparently the relative decrease of those that remain constant.

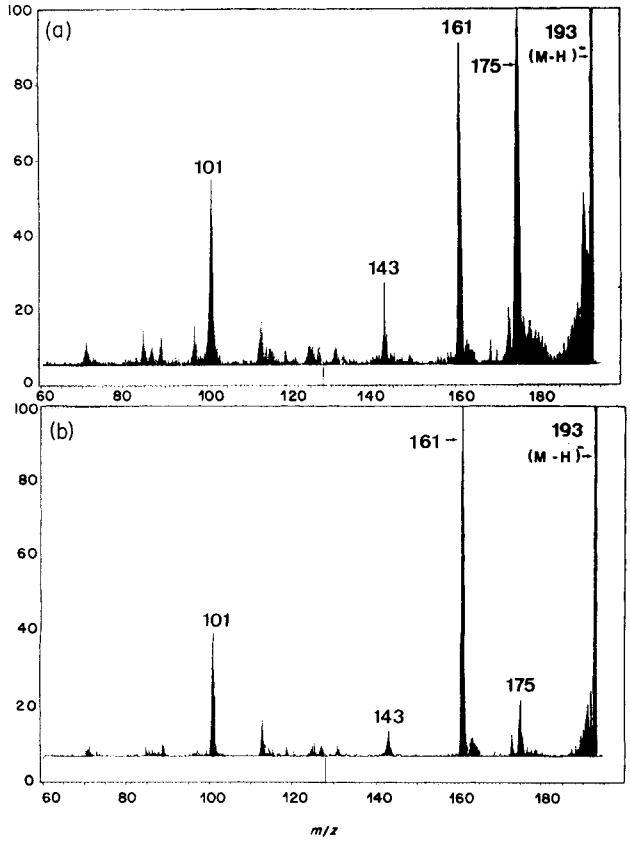


Figure 4 MIKE spectra of the $[M-H]^-$ ions of methyl-α-D-galactopyranoside (4(a)) and methyl-β-D-galactopyranoside (4(b)).

The present mechanistic proposal for the glycosidic cleavage of oxyanions of sugars appears to be consistent with our experimental results. It can also easily explain the MIKE spectra of the $[M-H]^-$ ions from several disaccharides. It should be noted that the opening of the sugar ring according to the first step of the present mechanism is similar to the stepwise decomposition pathway of oxyanions from polyethylene-glycols via the elimination of molecules of ethylene oxide.[12] However, other competitive pathways could also occur, depending on the structure of the molecule, and it was recently demonstrated that the cleavage of a cyclic ketal by alcoholate ions works through an hydride intramolecular attack. (J. C. Tabet, personal communication).

EXPERIMENTAL

Chemicals

The following methylglycopyranosides were purchased from Sigma and were of the best purity available: methyl-α-D-glucopyranoside, methyl-β-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-α-D-galactopyranoside, methyl-β-D-galactopyranoside, methyl-6-deoxy-α-D-glucopyranoside and methyl-6-deoxy-β-D-glucopyranoside.

The methyl-4,6-*O*-benzylidene-α-D-glucopyranoside and its β-anomer were synthesized from the corresponding methylglucopyranosides by well described methods.[13]

Methyl-2-*O*-trideuteriomethyl-α-D-glucopyranoside 3

Methyl-2-*O*-trideuteriomethyl-α-D-glucopyranoside was synthesized from methyl-2-*O*-palmitoyl-α-D-glucopyranoside, a derivative which was synthesized in a preceding work.[14] The method of synthesis was a slight modification of that already described.[14] Methyl-2-*O*-palmitoyl-α-D-glucopyranoside (8 mg) was dissolved in anhydrous diethylether (1 cm^3) and freshly distilled dihydropyran (0.2 cm^3). A 1% solution of paratoluene sulphonic acid in dry diethylether (0.2 cm^3) was then added and the mixture was stirred at ambient temperature for 0.5 h. After complete dissolution, 0.4 cm^3 of a 1M solution of dimsyl sodium in dry dimethylsuphoxide was added under a stream of nitrogen and the mixture was left to react for 2 h. Then, 0.3 cm^3 of trideuteriomethyl iodide was added and the mixture was left overnight. Selective hydrolysis of the tetrahydropyranyl groups was performed by addition of 5 cm^3 aqueous acetic acid (1:1, v/v). After 1 h at 100°C, the mixture was cooled, diluted with water (5 cm^3) and extracted two times with ether. The aqueous layer was desalted over a mixed bed resin (Amberlite Mb 3) and evaporated. The residue was purified by chromatography on Silicar CC7 (Mallinckrodt) using chloroform–methanol mixture. Positive FAB spectrum: MH^+ at m/z 212. The MIKE spectrum of m/z 212 showed an intense metastable transition at $E/Eo = 0.849$, corresponding to the fragment ion m/z 180.1 (loss of methanol).

The presence of the trideuteriomethyl group on C-2 was confirmed by the electron impact (EI) mass spectra of the corresponding alditol acetate. The 70 eV EI mass spectrum showed an intense peak at m/z 121 (80% R.I.) corresponding to the fragment $CD_3-\overset{+}{O}=CH-CHD-OAc$ and smaller fragments: m/z 336 (4%), $CD_3-\overset{+}{O}=CH-(CHOAc)_4-CH_2OH$; m/z 259 (2%), loss of ketene and trideuteriomethanol from m/z 336.

Methyl-4,6-*O*-trideuteriomethyl-β-D-glucopyranoside 5

Methyl-4,6-*O*-benzylidene-β-D-glucopyranoside was benzylated with benzyl chloride and potassium hydroxide in toluene.[15] The methyl-4,6-*O*-benzylidene-2,3-di-*O*-benzyl-β-D-glucopyranoside was purified by column chromatography (Silicar CC7) using a 95:5 hexane-ethyl acetate mixture. The field desorption mass spectra showed an intense peak at m/z 462 corresponding to M^+. The benzylidene group was removed by selective hydrolysis, in a boiling 4:3 mixture of acetic acid in water (1 h). After neutralization with sodium carbonate, the mixture was extracted with chloroform, evaporated and purified on Silicar CC7. The methyl-2,3-di-*O*-benzyl-β-D-glucopyranoside was eluted with chloroform. The field desorption mass spectrum showed the presence of $M^{+\cdot}$ (m/z 374), $[MH]^+$ (m/z 375) and $[M-H]^+$ (m/z 373) in the intensity ratio 1:5:2.

This product was trideuteriomethylated using NaH in dimethylsulphoxide, followed by the addition of trideuteriomethyl iodide. Hydrogenolysis of the benzyl group was performed in ethyl acetate using palladium on charcoal (10%) as catalyst. The methyl-4,6-di-*O*-trideuteriomethyl-β-D-glucopyranoside was purified on column chromatography (Silicar CC7) using a 9:1 chloroform/methanol mixture. The product showed a single spot on TLC plates and the trimethylsilyl-derivative produced a single peak on capillary gas chromatography. The EI mass spectra of the alditol acetate (reduction step: sodium borodeuteride) showed the following characteristic fragments: m/z 48 (20%), $CH_2=\overset{+}{O}-CD_3$; m/z 167 (20%), $CD_3-O-CH_2-CHOAc-CH=\overset{+}{O}-CD_3$; m/z 132 (35%), loss of deuterated methanol from m/z 167; m/z 107 (20%), loss of acid acetic from m/z 167; m/z 265 (3%), $CHD\,OAc-(CHOAc)_2-CH=\overset{+}{O}-CD_3$; m/z 205 (2%), loss of acetic acid from m/z 265. The positive FAB spectra of **5** showed an intense $[MH]^+$ ion at m/z 229 and a metastable decomposition $m/z\ 229^+ \rightarrow m/z\ 197^+$ (loss of methanol).

Trideuteriomethyl-4,6-di-*O*-methyl-α-D-glucopyranoside 6

The methyl-4,6-di-*O*-methyl-α-D-glucopyranoside was synthesized as for the preceding labelled compound, using unlabelled methyl iodide in the methylation step. The glycosidic methyl group was exchanged by a trideuteriomethyl group by refluxing for 2 h with a 3% solution of anhydrous DCl in CD_3OD (prepared by reaction of acetylchloride on tetradeuterated methanol). The positive FAB mass spectra showed the $[MH]^+$ ion at m/z 226 and a metastable decomposition $m/z\ 226^+ \rightarrow m/z\ 191^+$ (loss of trideuterated methanol). The EI mass spectra of alditol acetate (reduction step: sodium borohydride) showed the characteristic peaks of a 4,6-di-*O*-methyl-1,2,3,5-tetra-*O*-acetyl-1-*d*-hexitol: m/z 45 (23%), m/z 101 (20%), m/z 129 (40%), m/z 161 (20%), m/z 202 (3%) and m/z 262 (5%).

Mass Spectrometry

The mass spectrometer was a ZAB 2F (VG Instrument) equipped with a FAB source and the DS 2050 Data System. The MIKE spectra were measured using the MIKE software, at a scan speed of 10 s/decade over the whole energy range. According to the signal intensities, 16 to 48 successive scans were accumulated. The MIKE spectra were directly plotted as mass calculated spectra. The accelerating voltage was ±8 kV. The xenon beam from the FAB gun had an energy of 8 keV. The GC/MS experiments were done on the same instrument fitted with the EI. source at 70 eV. A 30 m × 0.32 mm OV-1 capillary column was used. Helium was the carrier gas. The spectra were recorded by the Data System. The scan speed of the magnet was 1 s/decade.

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