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Differentiation of Anomeric *C*-Glycosides by Mass Spectrometry Using Fast Atom Bombardment, Mass-analysed Ion Kinetic Energy and Collision-activated Dissociation

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Positive-ion fast atom bombardment mass spectrometry appears to be a useful method for the differentiation of anomeric *C*-glycosides. The mass-analysed ion kinetic energy (MIKE) and collision-activated dissociation (CAD) MIKE spectra of selected positive ions can be used as fingerprints of the α- or β-anomers. The main fragmentation routes and particularly the formation of the $[M - H]^+$ ion and the $[M + H - PhCH_2OH]^+$ ion were traced for each anomer.

INTRODUCTION

Fast atom bombardment (FAB) ionization has been shown to be a very useful method in the mass spectrometric analysis of a wide range of natural compounds, particularly carbohydrates.[1] However, although FAB itself provides relative molecular mass and some sequencing information, the low abundance of fragment ions and chemical noise from ions of the FAB matrix limit this sequence information. These disadvantages are overcome by isolating the molecular ion of interest from chemical noise or interferences. Thus, FAB combined with tandem mass spectrometry (MS/MS)[2] has been applied to the determination of the structures of polysaccharides and glycoconjugates and, in some cases, the differentiation of stereoisomeric sugars. FAB MS/MS and collision-activated dissociation (CAD) have been used to determine the sequence and the pattern of branching of oligosaccharides.[3–6] Collision activation and chemical ionization in mass-analysed ion kinetic energy (MIKES) spectra have also been used to discriminate among anomers of permethylated mono- and disaccharides.[7–13] FAB and CAD–MIKE techniques have also been used for the identification of aldohexoses,[14–16] stereoisomers of hexoses and other complex saccharides and nucleosides.[17–25]

Although FABMS has been used to analyse *C*-glycosidic flavonoids[26] and *C*-arylglycosides,[27] no example of the differentiation of anomeric *C*-glycosides has been reported. We have shown recently[28] that some anomeric unsaturated *C*-glycosides showed a distinct and discriminating ion pattern; the daughter-ion mass spectra of the two anomers had an ion abundance 'fingerprint' which was characteristic of each anomer and the sequential dissociation of these daughter ions to granddaughter ions resulted in further characteristic 'fingerprint' patterns which were unique to the molecular structure of the selected daughter ions. Here we report a more detailed FAB MIKE and FAB CAD–MIKE investigation of various pairs of anomeric unsaturated *C*-glycosides.

EXPERIMENTAL

The FAB mass spectra were recorded on a VG ZAB 2-SEQ mass spectrometer (Fisons, Manchester) fitted with a caesium ion gun delivering about 2 μA of caesium ion current with about 35 kV energy. All the samples were analysed by dissolving them in dichloromethane and mixing the solution (1–2 μl) into 1–2 μl of matrix (3-nitrobenzyl alcohol) already on the target. The FAB CAD–MIKE analyses were performed using helium as the collision gas at such a pressure that the intensity of the mass-selected beam was reduced by ~80% of its original value. The laboratory collision energy was 8 keV.

Compounds **1–4** (Scheme 1) were prepared as described previously.[29] Compounds **5** and **6** were obtained by hydrogenation of **1α** and **2**, respectively, in the presence of $RhCl(PPh_3)_3$ and compounds **7** by reduction of the corresponding benzylated unsaturated compounds[29] in the presence of Pd/C followed by acetylation. The products were carefully purified by column chromatography on silica gel.

Ethyl 2-(2′,3′-dideoxy-4′,6′-di-*O*-benzyl-α-D-glucopyranosyl-2-nitro-2-carboethoxy acetate (**5**). $[\alpha]_D^{20}$ +30° (*c* 1.3, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$), δ 1.29 (3H,

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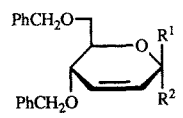
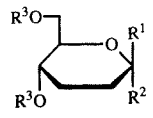




1α $R^1 = H, R^2 = C(NO_2)(CO_2C_2H_5)_2$
1β $R^1 = C(NO_2)(CO_2C_2H_5)_2, R^2 = H$
2α $R^1 = H, R^2 = CH(NO_2)CO_2C_2H_5$
2β $R^1 = CH(NO_2)CO_2C_2H_5, R^2 = H$
3α $R^1 = H, R^2 = C(COCH_3)_2(COOCH_3)$
3β $R^1 = C(COCH_3)_2(COOCH_3), R^2 = H$
4α $R^1 = H, R^2 = CH_2COOC_2H_5$
4β $R^1 = CH_2COOC_2H_5, R^2 = H$

5 $R^1 = H, R^2 = C(NO_2)(CO_2C_2H_5)_2, R^3 = CH_2Ph$
6α $R^1 = H, R^2 = CH(NO_2)CO_2C_2H_5, R^3 = CH_2Ph$
6β $R^1 = CH(NO_2)CO_2C_2H_5, R^2 = H, R^3 = CH_2Ph$
7α $R^1 = H, R^2 = CH(CO_2C_2H_5)_2, R^3 = COCH_3$
7β $R^1 = CH(CO_2C_2H_5)_2, R^2 = H, R^3 = COCH_3$

Scheme 1

t, Me), 1.30 (3H, t, Me), 2.10–2.30 [1H, m H(2′$_{ax}$)], 2.40–2.55 [2H, m, H(2′$_{eq}$), H(3′$_{ax}$)], 2.60–2.70 [1H, m, H(3′$_{eq}$)], 3.77 [1H, dd, $J_{6',6''} = 10.6$ Hz, $J_{6',5'} = 2.0$ Hz, H(6′)], 3.90 [1H, dd, $J_{6'',6'} = 10.6$ Hz, $J_{6'',5'} = 2.2$ Hz, H(6″)], 4.27–4.47 [5H, m, OCH_2, H(4′)], 4.5 [1H, ddd, $J_{5',6'} = 2.0$ Hz, $J_{5',6''} = 2.2$ Hz, $J_{5',4'} = 11.9$ Hz, H(5′)], 4.55 and 4.60 (2 × 1H, 2 × d, OCH_2Ph), 4.65 and 4.70 (2 × 1H, 2 × d, OCH_2Ph), 4.9 [1H, bs, H(1′)], 7.22–7.37 (10H, m, C_6H_5); ^{13}C NMR (75.45 MHz, $CDCl_3$), δ 13.77 (Me), 24.40 [C(2′)], 24.60 [C(3′)], 63.50 (OCH_2), 69.04 [C(6′)], 71.06 and 73.32 (OCH_2Ph), 71.29 [C(4′)], 74.29 [C(1′)], 92.69 [C(5′)], 98.72 [C(2)], 126.8–131.2 (C_6H_5), 160.92 and 161.18 ($COOC_2H_5$).

Ethyl 2-(2′,3′-dideoxy-4′,6′-di-*O*-benzyl-α-D-glucopyranosyl)-2-carboethoxy acetate (**6**). α-Anomer (as a 74:26 mixture of the two epimers at C-2): ^1H NMR (300 MHz, $CDCl_3$) δ 1.26 and 1.29 (0.26 × 3H and 0.74 × 3H, 2 × t, Me), 1.89 [4H, m, H(2′), H(3′)], 3.59 [1H, m, H(4′)], 3.65 [1H, dd, $J_{6',6''} = 13.3$ Hz, $J_{6',5'} = 4.8$ Hz, H(6′)], 3.68 (1H, dd, $J_{6'',6'} = 13.3$ Hz, $J_{6'',5'} = 4.7$ Hz, H(6″)], 3.89 [1H, ddd, $J_{5',6'} = 4.8$ Hz, $J_{5',6''} = 4.7$ Hz, $J_{5',4'} = 9.5$ Hz, H(5′)], 4.26 and 4.27 (0.26 × 2H and 0.74 × 2H, 2 × t, OCH_2), 4.54 (5H, m, H-1′, OCH_2Ph), 5.32 and 5.37 [0.26 × 1H and 0.74 × 1H, 2 × d, $J_{2,1'} = 9.0$ and 10.0 Hz, H(2)], 7.30 (10H, m, C_6H_5); ^{13}C NMR (75.45 MHz, $CDCl_3$), δ 14.21 (Me), 23.02 and 23.47 [0.74 and 0.26 C(2′)], 24.20 and 24.35 [0.26 and 0.74 C(3′)], 63.55 (OCH_2), 69.25 and 69.41 [0.26 and 0.74 C(6′)], 71.68 and 73.87 (OCH_2Ph), 71.84 and 71.99 [0.74 and 0.26 C(4′)], 75.28 and 75.54 [0.74 and 0.26 C(1′)], 81.57 and 81.95 [0.26 and 0.74 C(5′)], 88.12 and 89.58 [0.26 and 0.74 C(2)], 127.0–128.8 (C_6H_5), 162.57 ($COOC_2H_5$). β-Anomer [as a 66:34 mixture of the two epimers at C(2)]: ^1H NMR (300 MHz, $CDCl_3$), δ 1.26 and 1.29 (0.26 × 3H and 0.74 × 3H, 2 × t, Me), 1.52 [2H, m, H(2′$_{ax}$), H(3′$_{ax}$)], 1.89 [1H, m, H(2′$_{eq}$)], 2.32 [1H, m, H(3′$_{eq}$)], 3.41 [1H, ddd, $J_{5',6'} = 2.0$ Hz, $J_{5',6''} = 4.0$ Hz, $J_{5',4'} = 10.0$ Hz, H(5′)], 3.42 [1H, m, H(4′)], 3.62 [1H, dd, $J_{6',6''} = 10.0$ Hz, $J_{6',5'} = 2.0$ Hz, H(6′)], 3.70 [1H, dd, $J_{6'',6'} = 10.0$ Hz, $J_{6'',5'} = 4.0$ Hz, H(6″)], 4.16 and 4.21 [0.34 × 1H and 0.66 × 1H, m, H(1′)], 4.26 and 4.27 (0.34 × 2H and 0.66 × 2H, 2 × t, OCH_2), 4.51 (4H, m, OCH_2Ph), 5.12 and 5.17 [0.66 × 1H and 0.34 × 1H, 2 × d, $J_{2,1'} = 9.3$ and 6.7 Hz, H(2)], 7.30 (10H, m, C_6H_5); ^{13}C NMR (75.45 MHz, $CDCl_3$), δ 13.81 (Me), 26.42 and 26.62 [0.34 and 0.66 C(2′)], 28.58 and 28.63 [0.34 and 0.66 C(3′)], 63.17 (OCH_2), 68.84 and 68.99 [0.66 and 0.34 C(6′)], 71.26 and 73.34 (OCH_2Ph), 72.04 and 72.28 [0.66 and 0.34 C(4′)], 74.86 and 75.12 [0.34 and 0.66 C(1′)], 81.17 and 81.54 [0.66 and 0.34 C(5′)], 89.66 and 90.97 [0.34 and 0.66 C(2)], 127.5–128.4 (C_6H_5), 162.15 ($COOC_2H_5$).

Ethyl 2-(2′,3′-dideoxy-4′,6′-di-*O*-acetyl-D-glucopyranosyl)-2-carboethoxy acetate (**7**). α-Anomer: $[\alpha]_D^{20}$ +33° (*c* 0.5, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$), δ 1.27 (3H, t, Me), 1.29 (3H, t, Me), 1.70–1.90 [3H, m, H(2′) + H(3′$_{ax}$)], 1.95–2.05 [1H, m, H(3′$_{eq}$)], 2.07 (3H, s, OAc), 2.08 (3H, s, OAc), 3.74 [1H, d, $J_{2,1'} = 10.2$ Hz, H(2)], 3.96 [1H, ddd, $J_{5',6'} = 4.5$ Hz, $J_{5',6''} = 6.2$ Hz, $J_{5',4'} = 6.3$ Hz, H(5′)], 4.11 [1H, dd, $J_{6',6''} = 11.8$ Hz, $J_{6',5'} = 4.5$ Hz, H(6′)], 4.19 (2H, q, CH_2), 4.23 (2H, q, CH_2), 4.27 [1H, dd, $J_{6'',6'} = 11.8$ Hz, $J_{6'',5'} = 6.2$ Hz, H(6″)], 4.42 [1H, ddd, $J_{1',2} = 10.2$ Hz, $J_{1',2'ax} = 6.2$ Hz, $J_{1',2'eq} = 3.9$ Hz, H(1′)], 4.76 [1H, ddd, $J_{4',5'} = 6.3$ Hz, $J_{4',3'ax} = 5.8$ Hz, $J_{4',3'eq} = 3.9$, H(4′)]; ^{13}C NMR (75.45 MHz, $CDCl_3$), δ 13.97 and 14.02 (Me), 21.09 and 20.72 ($OCOCH_3$), 24.06 [C(3′)], 24.31 [C(2′)], 55.01 [C(2)], 61.53 and 61.60 (OCH_2), 62.21 [C(6′)], 67.08 [C(4′)], 70.35 [C(1′)], 72.33 [C(5′)], 167.1 and 166.7 ($COOC_2H_5$), 170.1 and 170.7 ($OCOCH_3$). β-Anomer: $[\alpha]_D^{20}$ +19.8° (*c* 0.5, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$), δ 1.27 (3H, t, Me), 1.28 [3H, t, Me), 1.50–1.60 (3H, m, H(2′$_{ax}$) + H(3′$_{ax}$)], 1.90–1.97 [1H, m, H(2′$_{eq}$)], 2.04 (6H, s, 2 × OAc), 2.20–2.30 [1H, m, H(3′$_{eq}$)], 3.46 [1H, d, $J_{2,1'} = 9.5$ Hz, H(2)], 3.58 [1H, ddd, $J_{5',6'} = 2.5$ Hz, $J_{5',6''} = 5.8$ Hz, $J_{5',4'} = 9.8$ Hz, H(5′)], 4.02 [1H, ddd, $J_{1',2} = 9.5$ Hz, $J_{1',2'ax} = 11.2$ Hz, $J_{1',2'e} = 2.4$ Hz, H(1′)], 4.08 [1H, dd, $J_{6',6''} = 12.1$ Hz, $J_{6',5'} = 2.5$ Hz. H(6′)], 4.18 [1H, dd, $J_{6'',6} = 12.1$ Hz, $J_{6'',5'} = 5.8$ Hz, H(6″)], 4.19 (2H, q, CH_2), 4.21 (2H, q, CH_2), 4.65 [1H, ddd, $J_{4',5'} = 9.8$ Hz, $J_{4',3'ax} = 10.4$ Hz, $J_{4',3'eq} = 4.2$ Hz, H(4′)]; ^{13}C NMR (75.45 MHz, $CDCl_3$), δ 13.97 and 14.02 (Me), 20.70 and 20.99 ($OCOCH_3$), 28.07 [C(2′)], 28.73 [C(3′)], 57.33 [C(2)], 61.43 and 61.59 (OCH_2), 63.27 [C(6′)], 67.71 [C(4′)], 75.78 [C(1′)], 77.70 [C(5′)], 166.6 and 167.1 ($COOC_2H_5$), 169.9 and 170.7 ($OCOCH_3$).

RESULTS AND DISCUSSION

A series of anomeric pairs of unsaturated and saturated *C*-glycosides, summarized in Scheme 1, were investigated by the FAB method. The sigificant ions observed in the positive-ion FAB mass spectra of these compounds are presented in Table 1, where the origin of individual ions is also indicated.

Table 1. Significant ions observed in the positive-ion FAB mass spectra of compounds 1–7

	m/z (relative intensity, %)									
Compound	$[M+H]^+$	$[M-H]^+$	$[M+H-HNO_2]^+$	$[M+H-PhCH_2OH]^+$	$[M+H-HR^1R^2]^+$	$[M+H-2PhCH_2OH]^+$	$[M+H-(PhCH_2)O]^+$	$[M+H-HR^1R^2-PhCH_2OH]^+$	$PhCH_2C^+HPh$	Others
1α	514 (12)	512 (18)	467 (11)	406 (71)	309 (6)	298 (28)	316 (10)	201 (100)	(64)	206 (10)[a]
1β	514 (43)	512 (35)	467 (13)	406 (71)	309 (12)	298 (6)	316 (23)	201 (68)	(100)	206 (19)[a]
2α	442 (16)	440 (45)	395 (0)	334 (55)	309 (8)	226 (16)	244 (23)	201 (30)	(100)	287 (10)[b]
2β	442 (25)	440 (38)	395 (6)	334 (24)	309 (4)	226 (8)	244 (21)	201 (27)	(100)	287 (5)[b]
3α	467 (3)	465 (11)		359 (28)	309 (13)	251 (5)	269 (5)	201 (25)	(100)	423 (20)[c]
										299 (29)[d]
										267 (38)[e]
3β	467 (0)	465 (53)		359 (44)	309 (62)	251 (0)	269 (0)	201 (22)	(100)	423 (49)[c]
										267 (27)[e]
4α	397 (44)	395 (19)		289 (100		181 (87)	199 (8)	201 (6)	(87)	
4β	397 (60)	395 (27)		289 (82)		181 (100)	199 (23)	201 (8)	(100)	
5α	516 (1)	514 (2)							(100)	206 (19)[a]
6α	444 (8)	442 (34)	397 (2)	336 (2)		228 (6)		203 (5)	(100)	
6β	444 (3)	442 (22)	397 (6)	336 (0)		228 (7)		203 (4)	(100)	
7α	375 (100)			315 (61)[f]	215 (47)	255 (21)[g]				161 (35)[a]
7β	375 (100)			315 (62)[f]	215 (50)	255 (38)[g]				161 (36)[a]

[a] $[R^1, R^2+H]^+$.
[b] $[M+H-PhCH_2OH-HNO_2]^+$.
[c] $[M+H-CH_3CHO]^+$.
[d] $[M+H-PhCH_2OH-HCO_2Me]^+$.
[e] $[M+H-PhCH_2OH-PhMe]^+$.
[f] $[M+H-CH_3COOH]^+$.
[g] $[M+H-2\times CH_3COOH]^+$.

It can be seen from the FABMS of unsaturated *C*-glycosides **1**–**4** that only **1**, **2**, and **4** exhibit protonated molecular ions $[M+H]^+$, in higher abundance for the β-anomer. Compound **3** gives rise to relatively low-abundant $[M+H]^+$ ion only for the α-anomer (less than 3%). We observe the presence of the ions $[M-H]^+$ for all the compounds, with generally a higher abundance for the β-anomer, except for **2**. The most important fragmentation of the $[M+H]^+$ ion is the loss of one molecule of benzyl alcohol ($[M+H-PhCH_2OH]^+$) (base peak for **4α**), eventually followed by the cleavage of the aglycone moiety leading to the ion at *m/z* 201 ($[M+H-PhCH_2OH-R^1R^2H]^+$) (base peak for **1α**). For all compounds, except **1α** and **4α**, the base peak is the ion at *m/z* 191 ($PhCH_2C^+HPh$). We also observe some minor fragmentations corresponding to the loss of two molecules of benzyl alcohol, of $(PhCH_2)_2O$, of the aglycone moiety (*m/z* 309) and for the nitro compounds the cleavage of HNO_2.

The FABMS of saturated *C*-glycosides **5** and **6** shows only minute amounts of the $[M+H]^+$ ion. We observe also the presence of the $[M-H]^+$ ion, important for compounds **6**. For these compounds the base peak is $PhCH_2C^+HPh$ (*m/z* 191).

The saturated acetylated *C*-glycosides **7α** and **7β** show different FAB mass spectra. We only observe the protonated molecular ion at *m/z* 375 (base peak for the two anomers) and no ion at *m/z* 373. The most important fragment ions at *m/z* 315, 255, 215 and 161 correspond to a single loss of CH_3COOH, a double loss of CH_3COOH, a loss of $CH_2(NO_2)CO_2C_2H_5$ from the protonated molecular ion and to the $[CH_2(NO_2)CO_2C_2H_5+H]^+$ ion, respectively.

In conclusion, although some peaks can barely be distinguished from the background and also taking into account the lower reproductibility of FAB mass spectra, it is evident that some small differentiations appear between the α- and β-anomeric *C*-glycosides. The most important fact is the presence of the $[M-H]^+$ ion for the benzylated *C*-glycosides, which is absent for the acetylated *C*-glycosides. To obtain more information about the structure of the $[M-H]^+$ ions and to investigate the possible differentiation of the anomers, we examined these compounds in detail using the MIKE method.

FAB MIKE and FAB CAD–MIKE spectra of $[M+H]^+$ ions

The respective FAB MIKE and FAB CAD–MIKE spectra of the pairs of anomers were compared in order to obtain possible reliable differences that would allow the characterization of these anomers. The relative abundances of the major daughter ions in FAB MIKE and FAB CAD–MIKE spectra are listed in Tables 2 and 3.

The MIKE and CAD–MIKE spectra of anomeric **1α** and **1β** (Fig. 1) display large variations in the relative

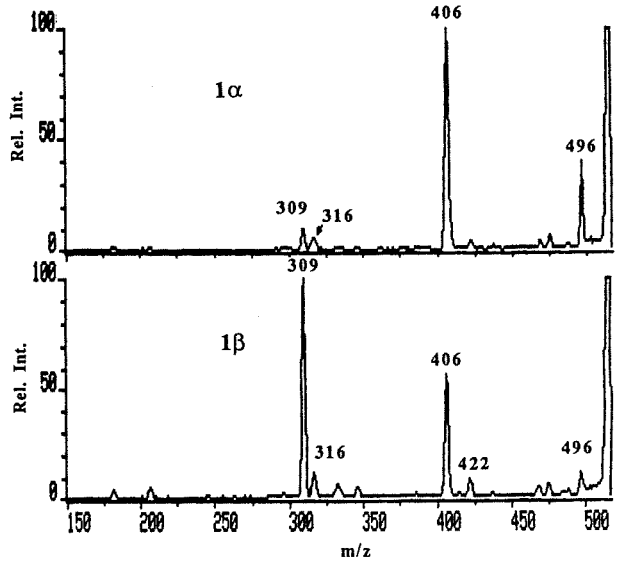
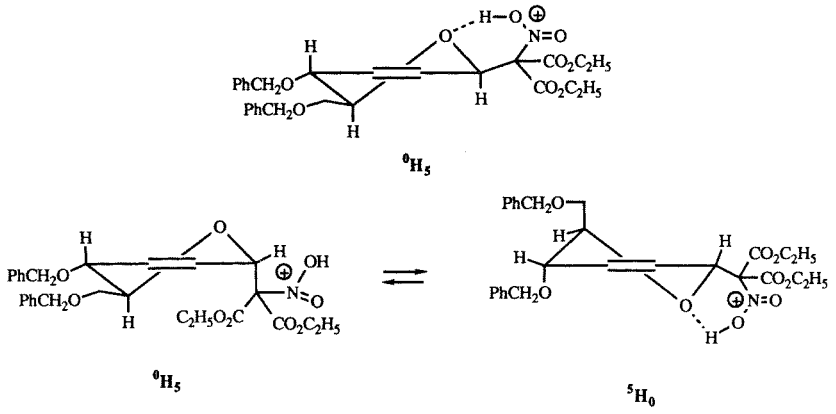


Figure 1. FAB MIKE spectra of the *m/z* 514 ($[M+H]^+$) ion of **1α** and **1β**.



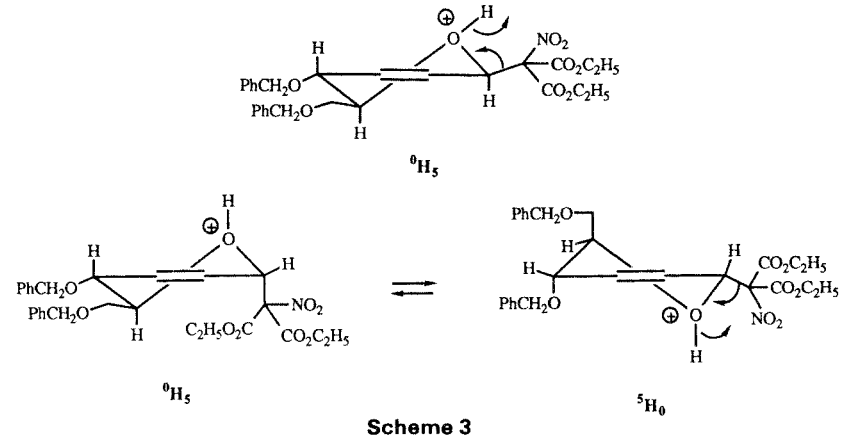
Scheme 2

abundances of daughter ions. The $[M + H]^+$ ion can readily lose water to form the $[M + H - H_2O]^+$ ion at *m/z* 496. The lower abundance of this ion in the β-anomer is probably correlated with the conformational equilibrium (Scheme 2); in the β-anomer, which is expected to exist only in the $^0H_5$ form, hydrogen bonding between the oxygen ring and the protonated oxygen (of the nitro group or the ester function) lowers the probability of losing H_2O. For the two anomers we observe a very important fragmentation at *m/z* 406 resulting from the cleavage of a molecule of benzyl alcohol from the $[M + H]^+$ ion (base peak for the α-anomer). However, the base peak at *m/z* 309 for the β-anomer results from the cleavage of the aglycone moiety; this fragment is also observed for the α-anomer but in very low abundances. This difference between the two anomers could be related to the configuration of the anomeric centre; for the β-anomer (Scheme 3), the proton associated with the ring oxygen and the aglycone are in a *syn* relationship, which favours the elimination of the aglycone moiety; this is not the case for the α-anomer. We also observe for the β-anomer the losses of $PhCH_3$ and $(PhCH_2)_2O$, giving the ions at *m/z* 422 and 316, respectively, and in the FAB CAD–MIKE spectra the presence of $[C_7H_7]^+$ at *m/z* 91 in very high abundance for the β-anomer.

The FAB MIKE and FAB CAD–MIKE spectra of **5**, the saturated analogue of **1α**, show some of the above fragmentations. The loss of water from the $[M + H]^+$ ion giving the ion at *m/z* 498 is again very important. We also observe the fragment ion at *m/z* 334 (base peak in the FAB MIKE spectrum) arising from the successive loss of $PhCH_2OH$ and $HCO_2C_2H_5$, and fragmentations of C_2H_4 (*m/z* 488), C_2H_2O (*m/z* 474), $PhCH_3$ (*m/z* 424), $PhCH_2OH$ (*m/z* 408) and $(PhCH_2)_2O$ (*m/z* 318).

The FAB CAD and FAB CAD–MIKE spectra of the anomeric pair **2α** and **2β** show some similarities with the spectra of **1α** and **1β**. The base peak for the two anomers in the FAB CAD spectra is the ion at *m/z* 334 obtained by the loss of $PhCH_2OH$ from the $[M + H]^+$ ion. In the FAB CAD–MIKE spectra, however, if the base peak of the α-anomer is again *m/z* 334, for the β-anomer it is now the ion $[C_7H_7]^+$ at *m/z* 91. The loss of water from the $[M + H]^+$ ion affording the ion at *m/z* 424 is more important for the α-anomer, and the loss of the aglycone moiety leading to the ion at *m/z* 309 is found only for the β-anomer. These differences could again be related to the anomeric configuration. The other fragmentations are the loss of $HCO_2C_2H_5$, $PhCH_3$, $(PhCH_2)_2O$ and $[PhCH_2OH + HCO_2C_2H_5]$ from the $[M + H]^+$ ion giving the ions at *m/z* 368, 350, 244 and 260, respectively, in similar abundances for the two anomers.

The FAB CAD and FAB CAD–MIKE spectra of the *C*-glycosides **6α** and **6β**, saturated analogues of **2α** and **2β**, also show different fragmentations. In the FAB CAD spectra, the base peak for the α-anomer is the ion at *m/z* 264 arising from the loss of $[PhCHO + HCO_2C_2H_5]$ from the $[M + H]^+$ ion, whereas for the β-anomer it is the ion at *m/z* 334 obtained by the loss of $PhCH_3$ and H_2O from the $[M + H]^+$ ion. In the case of the β-anomer we observe also the very abundant



Scheme 3

Table 2. Principal daughter ions observed in the FAB MIKE spectra of the $[M + H]^+$ ion of compounds 1–7

	m/z (relative intensity, %)						
Compounds	$[M+H-H_2O]^+$	$[M+H-PhCH_3]^+$	$[M+H-PhCH_2OH]^+$	$[M+H-HR^1R^2]^+$	$[M+H-(PhCH_2)_2O]^+$	$PhCH_2C^+HPh$	Others
1α	496 (40)	422 (0)	406 (100)	309 (10)	316 (0)		
1β	496 (10)	422 (8)	406 (55)	309 (100)	316 (13)		
2α	424 (65)	350 (12)	334 (100)	309 (0)	244 (20)	181 (12)	403 (35)[a]; 368 (15)[b]
2β	424 (45)	350 (25)	334 (100)	309 (38)	244 (38)	181 (18)	403 (25)[a]; 368 (10)[b]; 260 (30)[c]
4α	379 (0)		289 (100)				
4β	379 (15)		289 (100)				
5	498 (85)	424 (28)	408 (50)	309 (25)	318 (40	181 (20)	488 (35)[d]; 474 (30)[e]; 390 (35)[f]; 334 (100)[c]
6α	426 (25)	352 (25)		311 (15)		181 (45)	338 (30)[g]; 276 (20)[h]; 264 (100)[i]
6β	426 (30)	352 (0)		311 (0)		181 (0)	397 (95)[j]; 334 (100)[k]
7α			315 (100)[l]				
7β			315 (100)[l]				

[a] $[M+H-39]^+$.
[b] $[M+H-HCO_2C_2H_5]^+$.
[c] $[M+H-PhCH_2OH-HCO_2C_2H_5]^+$.
[d] $[M+H-C_2H_4]^+$.
[e] $[M+H-C_2H_2O]^+$.
[f] $[M+H-H_2O-PhCH_2OH]^+$.
[g] $[M+H-PhCHO]^+$.
[h] $[M+H-PhCHO-NO_3]^+$.
[i] $[M+H-PhCHO-HCO_2C_2H_5]^+$.
[j] $[M+H-HNO_2]^+$.
[k] $[M+H-PhCH_3-H_2O]^+$.
[l] $[M+H-CH_3COOH]^+$.

ion at *m/z* 397 due to the loss of HNO_2 from the protonated molecular ion $[M + H]^+$, absent for the α-anomer. In the FAB CAD–MIKE spectra, if the α-anomer shows only two abundant fragments at *m/z* 181 ($PhCH_2C^+HPh$) and *m/z* 91 $[C_7H_7]^+$, the fragmentation of the β-anomer is more important; the base peak is the ion at *m/z* 397 (loss of HNO_2) and we observe two major fragments at *m/z* 334 (loss of $PhCH_3$ followed by H_2O) and *m/z* 91 ($[C_7H_7]^+$) and two other fragments at *m/z* 304 (loss of $PhCH_3$ followed by HNO_2) and *m/z* 181. Hence these anomeric saturated *C*-glycosides again show a different fragmentation

Table 3. Principal daughter ions observed in the FAB CAD–MIKE spectra of the $[M + H]^+$ ion of compounds 1–7

	m/z (relative intensity, %)							
Compound	$[M+H-H_2O]^+$	$[M+H-PhCH_3]^+$	$[M+H-PhCH_2OH]^+$	$[M+H-(PhCH_2)_2O]^+$	$[M+H-HR^1R^2]^+$	$PhCH_2C^+HPh$	$C_7H_7^+$	Others
1α	496 (15)	422 (0)	406 (100)	316 (0)	309 (10)	181 (0)	91 (10)	201 (10)[a]
1β	496 (0)	422 (15)	406 (95)	316 (30)	309 (100)	181 (30)	91 (75)	201 (15)[a]
2α	424 (15)	350 (10)	334 (100)	244 (10)	309 (0)	181 (30)	91 (60)	
2β	424 (10)	350 (25)	334 (60)	244 (10)	309 (14)	181 (65)	91 (100)	260 (19)[b]
4α			289 (100)			181 (15)	91 (20)	
4β			289 (100)			181 (15)	91 (35)	282 (50)[c]
5	498 (50)	424 (50)	408 (50)	318 (50)		181 (100)	91 (70)	334 (50)[b]
6α		352 (30)				181 (70)	91 (100)	338 (10)[d]; 276 (30)[e]; 264 (30)[f]
6β		352 (0)				181 (35)	91 (90)	397 (100)[g]; 334 (75)[h]; 304 (?)[i]
7α			315 (100)[j]		215 (30)			255 (20)[k]; 161 (15)[l]
7β			315 (100)[j]		215 (30)			255 (20)[k]; 161 (15)[l]

[a] $[M+H-PhCH_2OH-R^1,R^2]^+$.
[b] $[M+H-PhCH_2OH-HCO_2C_2H_5]^+$.
[c] $[M+H-CH_2CO_2C_2H_5-CO]^+$.
[d] $[M+H-PhCHO]^+$.
[e] $[M+H-PhCHO-NO_3]^+$.
[f] $[M+H-PhCHO-HCO_2C_2H_5]^+$.
[g] $[M+H-HNO_2]^+$.
[h] $[M+H-PhCH_3-H_2O]^+$.
[i] $[M+H-PhCH_3-HNO_2]^+$.
[j] $[M+H-CH_3COOH]$.
[k] $[M+H-2\times CH_3COOH]^+$.
[l] $[R^1,R^2+H]^+$.

pattern which is related to the anomeric configuration.

The FAB CAD and FAB CAD–MIKE spectra of the unsaturated *C*-glycosides **4α** and **4β** show virtually no differentiation. The FAB CAD spectra of the two anomers are identical with only the ion at *m/z* 289 corresponding to the loss of $PhCH_2OH$ from the $[M + H]^+$ ion. In the FAB CAD–MIKE spectra, if the base peak is again due to the ion at *m/z* 289, for the *β*-anomer we only observe an important fragmentation corresponding to the losses of $CH_2CO_2C_2H_5$ and CO (ion at *m/z* 282).

Finally, the FAB CAD and FAB CAD–MIKE spectra of the saturated *C*-glycosides **7α** and **7β** show no differentiation. We only observe one peak in the FAB CAD spectra at *m/z* 315 corresponding to the loss of acetic acid from the $[M + H]^+$ ion. The FAB CAD–MIKE spectra show, in addition to the peak at *m/z* 315, three minor peaks at *m/z* 255 and 215, arising from the loss of two molecules of acetic acid and the aglycone moiety, respectively, and at *m/z* 161, corresponding to the protonated aglycone moiety.

FAB MIKE and FAB CAD–MIKE spectra of $[M - H]^+$ ions

One of the most important features of the FAB positive-ion mass spectra of unsaturated *C*-glycosides **1–4** is the presence of the abundant ion $[M - H]^+$. It is very important to know its origin and also to consider differentiation between the ions $[M - H]^+$ arising from a pair of anomers (Tables 4 and 5).

First, we observe the $[M - H]^+$ ion only for the unsaturated and saturated benzylated *C*-glycosides. This implies that the loss of H_2 from the protonated molecular ion or the abstraction of a hydride ion from the neutral molecule probably occurs from the benzyloxy group and not from the ring.

In the FAB MIKE spectra of the $[M - H]^+$ ion of **1** (Fig. 2) we observe only one peak for the α-anomer, at *m/z* 406, obtained by the loss of PhCHO. The fragmentation of the *β*-anomer is more important with, in addition to the peak at *m/z* 406, peaks at *m/z* 465 (loss of HNO_2), *m/z* 420 (loss of $PhCH_3$), *m/z* 344 (loss of $PhCH_2NO_2H + CH_2O$), *m/z* 307 (loss of the aglycone moiety), *m/z* 206 (protonated aglycone residue) and *m/z* 181 ($PhCH_2C^+HPh$). The FAB CAD–MIKE spectrum of the α-anomer shows again the base peak at *m/z* 406

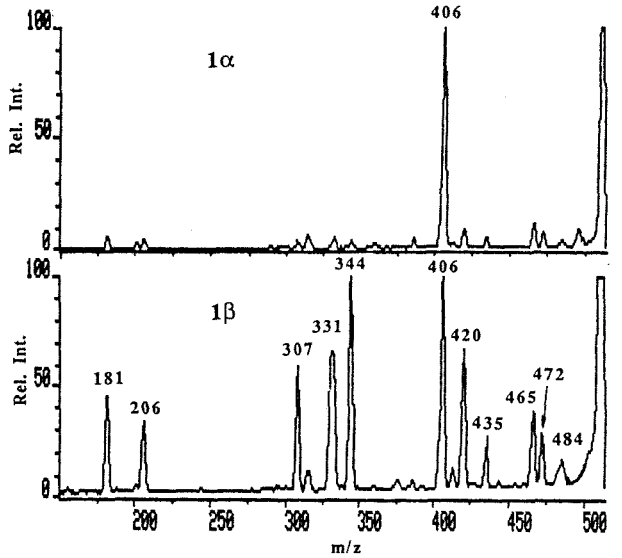


Figure 2. FAB MIKE spectra of the *m/z* 512 ($[M - H]^+$) ion of **1α** and **1β**.

Table 4. Principal daughter ions observed in the FAB MIKE spectra of the $[M - H]^+$ ion (= *a*) of compounds 1–6

	m/z (relative intensity, %)								
Compound	$a - H_2O$	$a - HNO_2$	$a - C_2H_5OH$	$a - PhCH_3$	$a - PhCHO$	$a - PhCH_2NO_2H - CH_2O$	$a - (PhCH_2)_2O$	$PhCH_2C^+HPh$	Others
1α		456 (0)		420 (0)	406 (100)	344 (0)		181 (0)	
1β		465 (38)		420 (65)	406 (100)	344 (95)		181 (45)	331 (65)[a]; 307 (60)[b]; 206 (35)[c]
2α				348 (5)	334 (100)	272 (0)		181 (10)	424 (10)[d]; 366 (6)[e]; 260 (10)[f]
2β				348 (40)	334 (100)	272 (45)		181 (60)	424 (65)[d]; 366 (25)[e]; 260 (55)[f]
3α	447 (20)		433 (32)[g]	373 (10)	359 (30)				423 (100)[h]; 405 (33)[i]
3β	447 (20)		433 (30)[g]	373 (32)	359 (12)				423 (85)[h]; 405 (100)[i]
4α	377 (100)		349 (35)	303 (20)			197 (15)		287 (100)[j]; 245 (20)[k]
4β	377 (100)		349 (20)	303 (15)			197 (0)		287 (30)[j]
6α	424 (0)	395 (10)		350 (35)	336 (100)	274 (22)	244 (12)	181 (25)	203 (12)[l]
6β	424 (50)	395 (70)		350 (35)	336 (100)	274 (0)	244 (0)	181 (0)	

[a] $[M - H - PhCH_2C^+HPh]$.
[b] $[M - H - HR^1R^2]^+$.
[c] $[R^1R^2H + H]^+$.
[d] $[M - H - O]$.
[e] $[M - H - HCO_2C_2H_5]^+$.
[f] $[M - H - PhCHO - HCO_2C_2H_5]^+$.
[g] Loss of CH_3OH.
[h] $[M - H - C_2H_2O]^+$.
[i] $[M + H - HCO_2CH_3]^+$.
[j] $[M - H - PhCH_2OH]^+$.
[k] $[M - H - PhCH_2OH - C_2H_2O]^+$.
[l] $[M - H - PhCHO - HR^1R^2]^+$.

Table 5. Principal daughter ions observed in the FAB CAD–MIKE spectra of the $[M - H]^+$ ion (= *a*) of compounds 1–6

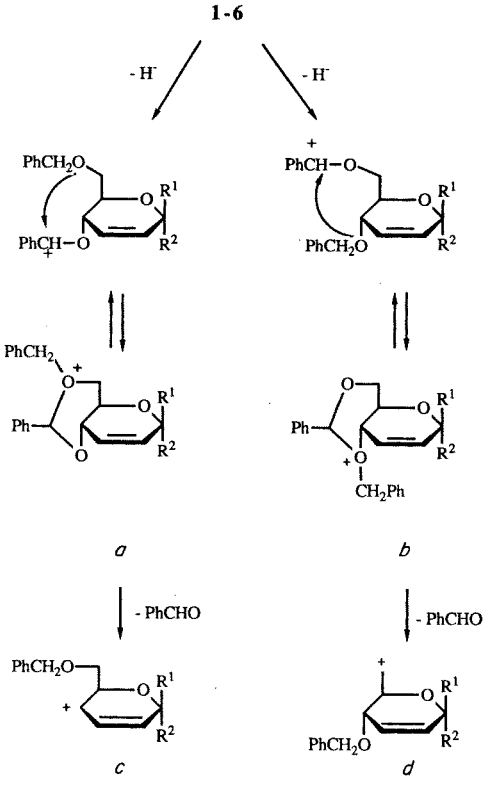
	m/z (relative intensity, %)							
Compound	$a - H_2O$	$a - C_2H_5OH$	$a - PhCH_3$	$a - PhCHO$	$a - PhCH_2NO_2H - CH_2O$	$PhCH_2C^+HPh$	$[C_7H_7]^+$	Others
1α	494 (50)		420 (0)	406 (100)	344 (0)	181 (0)	91 (0)	
1β	494 (0)		420 (35)	406 (20)	344 (30)	181 (75)	91 (100)	307 (20)[a]
2α			348 (30)	334 (75)	272 (10)	181 (70)	91 (100)	260 (10)[b]
2β			348 (30)	334 (25)	272 (20)	181 (70)	91 (100)	260 (15)[b]
3α		433 (35)[c]	373 (15)	359 (15)		181 (15)	91 (48)	423 (100)[d]; 405 (25)[e]; 391 (50)[f]
3β		433 (30)[c]	373 (42)	359 (10)		181 (45)	91 (92)	423 (100)[d]; 405 (45)[e]; 391 (40)[f]
4α	377 (80)	349 (50)	303 (30)			181 (38)	91 (60)	287 (100)[g]; 245 (20)[h]; 197 (10)[i]
4β	377 (100)	349 (48)	303 (20)			181 (20)	91 (35)	287 (60)[g]
6α	424 (0)		350 (30)	336 (62)	274 (12)	181 (35)	91 (100)	203 (18)[j]
6β	424 (35)		350 (35)	336 (100)	274 (20)	181 (30)	91 (92)	395 (60)[k]; 305 (40)[l]

[a] $[M - H - HR^1R^2]^+$.
[b] $[M - H - PhCHO - HCO_2C_2H_5]^+$.
[c] Loss of CH_3OH.
[d] $[M - H - C_2H_2O]^+$.
[e] $[M - H - HCO_2CH_3]^+$.
[f] $[M - H - MeOH - C_2H_2O]^+$.
[g] $[M - H - PhCH_2OH]^+$.
[h] $[M - H - PhCH_2OH - C_2H_2O]^+$.
[i] $[M - H - (PhCH_2)_2O]^+$.
[j] $[M - H - PhCHO - HR^1R^2]^+$.
[k] $[M - H - HNO_2]^+$.
[l] $[M - H - PhCH_3 - NO_2]$.

and a second fragmentation peak at *m/z* 494 (loss of water); even the β-anomer shows the same fragmentation as in FAB MIKE spectrum, the base peak now being at *m/z* 91 ($[C_7H_7]^+$). Thus we found some common trends between these spectra and the FAB spectra of the $[M + H]^+$ ion, with in particular the cleavage of the aglycone moiety being easier for the β-anomer.

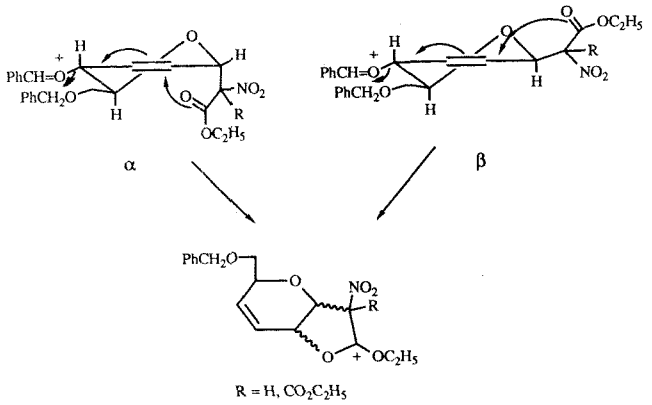
The FAB MIKE and FAB CAD–MIKE spectra of the ions $[M - H]^+$ derived from the two anomers being different, these ions are diastereoisomers to which we assign structures *a* and *b* (Scheme 4). As these ions are not seen in the MIKE spectra of $[M + H]^+$, they are probably formed in the condensed phase by abstraction of a hydride ion from the benzylic position giving $[M - H]^+$; these ions *a* and *b* can easily lose one molecule of PhCHO (base peak) leading to the ions *c* or *d*. The formation of only one peak at *m/z* 406 in the case of the α-anomer arising from the loss of PhCHO could be due to the participation of the aglycone moiety in the loss of PhCHO. Effectively, as shown in Scheme 5, the carboethoxy function could assist the cleavage of benzaldehyde leading to a bicyclic structure. In the case of the β-anomer, the configuration at the anomeric centre and the conformations of the molecule show that this assistance is less easy, leading to other possible fragmentations.

The FAB MIKE and FAB CAD–MIKE spectra of the $[M - H]^+$ ion of **2α** and **2β** are also different. In the FAB MIKE spectrum of **2α** the main fragmentation leads to the ion at *m/z* 334 arising from the loss of PhCHO from the $[M - H]^+$ ion; this important fragmentation could also be explained by the participation of the carboethoxy moiety in the cleavage of the PhCHO residue. For the β-anomer we observe the same



Scheme 4

base peak at *m/z* 334, and also some important fragmentations at *m/z* 424 (loss of an oxygen radical), *m/z* 348 (loss of $PhCH_3$), *m/z* 366 (loss of $HCO_2C_2H_5$), *m/z* 272 (loss of $PhCH_2NO_2H + CH_2O$), *m/z* 260 (loss of PhCHO and $HCO_2C_2H_5$) and *m/z* 181 ($PhCH_2C^+HPh$). The FAB CAD–MIKE spectra give



Scheme 5

no more information, the base peak being at *m/z* 91 ($[C_7H_7]^+$).

The FAB MIKE and FAB CAD–MIKE spectra of the $[M - H]^+$ ion of **3α** and **3β** are different from those of **1** and **2**. For the FAB MIKE spectra (Fig. 3) the base peak is at *m/z* 423 (loss of CH_2CO) for the α-anomer and at *m/z* 405 (loss of HCO_2CH_3) for the β-anomer. We also observe in similar amounts for the two anomers the fragment ions at *m/z* 447 (loss of H_2O), *m/z* 433 (loss of CH_3OH), *m/z* 373 (loss of $PhCH_3$) and *m/z* 359 (loss of PhCHO). The FAB CAD–MIKE spectra show only small differences, the base peak being at *m/z* 423 for the two anomers.

The FAB MIKE and FAB CAD–MIKE spectra of the $[M - H]^+$ ion of **4α** and **4β** are different. In the FAB MIKE spectra **4α** shows two base peaks at *m/z* 377 (loss of H_2O) and *m/z* 287 (loss of $PhCH_2OH$), whereas the β-anomer shows only the base peak at *m/z* 377, the peak at *m/z* 287 being of lower intensity. We also observe the loss of C_2H_5OH (*m/z* 349), $PhCH_3$ (*m/z* 303), and $PhCH_2OH$ followed by CH_2CO for the α-anomer (*m/z* 245). The FAB CAD–MIKE spectra show some similarities with two abundant fragments at *m/z* 377 (base peak for the β-anomer) and *m/z* 287 (base peak for the α-anomer). However, the FAB MIKE or

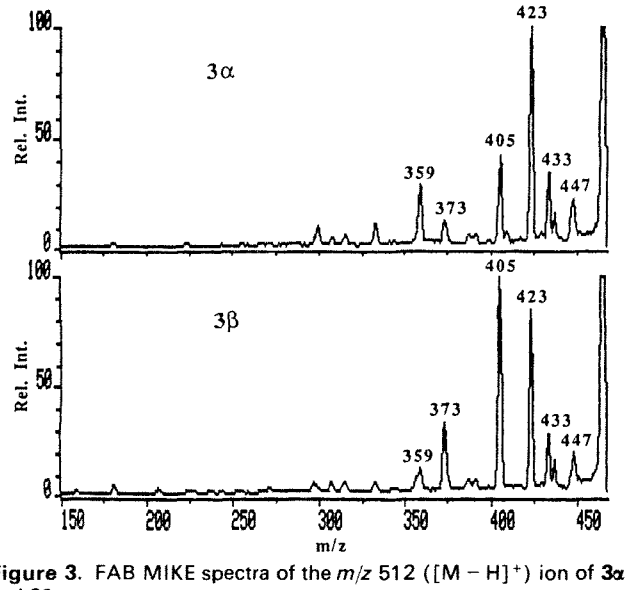


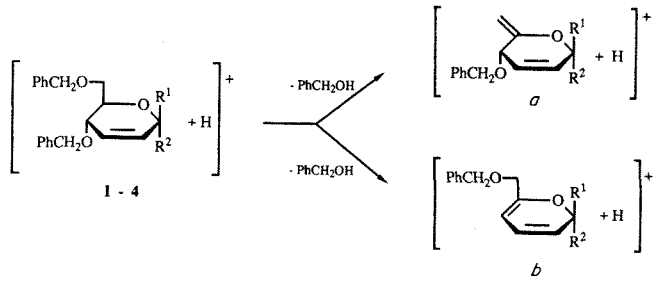
Figure 3. FAB MIKE spectra of the *m/z* 512 ($[M - H]^+$) ion of **3α** and **3β**.

the FAB CAD–MIKE spectra for the two anomers are different and are 'fingerprints' of these compounds.

For the saturated *C*-glycosides **6α** and **6β** we observe in the FAB MIKE spectra of the $[M - H]^+$ ion a very important fragment ion at *m/z* 336 (base peak) arising from the loss of PhCHO. We observe the loss of water (peak at *m/z* 424) for the β-anomer only, and the very important loss of HNO_2 for the β-anomer (*m/z* 395). The same typical fragmentations are found in the FAB CAD–MIKE spectra although the major ion is now $[C_7H_7]^+$ for the α-anomer.

FAB MIKE spectra of $[M + H - PhCH_2OH]^+$ ion

We observed in the FAB spectra of **1–4** a very important fragment ion arising from the loss of $PhCH_2OH$ from the $[M + H]^+$ ion. There are two possibilities for the loss of one molecule of benzyl alcohol leading to different daughter ions (Scheme 6). The loss of the secondary benzyloxy group from the α- and β-anomers would afforded two enantiomeric daughter ions *b* indistinguishable by mass spectrometry, whereas the loss of the primary benzyloxy group would afford two diastereoisomeric daughter ions *a* which could be differentiated by mass spectrometry. In order to obtain more information about the loss of $PhCH_2OH$, we studied the decomposition of the ion obtained in the FAB spectra of **2** and **3** by the loss of $PhCH_2OH$ by the MIKE technique (Table 6).



Scheme 6

For **2** the FAB MIKE spectra of the ion at *m/z* 334 (loss of $PhCH_2OH$) shows the fragment ions at *m/z* 318 (loss of an oxygen radical), *m/z* 287 (loss of HNO_2), *m/z* 228 (loss of PhCHO) and *m/z* 201 (loss of the aglycone moiety). The base peak is at *m/z* 228 for the α-anomer and *m/z* 201 for the β-anomer.

Table 6. Principal daughter ions observed in the FAB MIKE spectra of the $[M + H - PhCH_2OH]^+$ ion of compounds 2 and 3

m/z	2α	2β	3α	3β
331			30	35
330			30	35
318	45	42		
317			25	100
299			100	70
287	60	30		
267			15	15
228	100	70		
201	85	100		

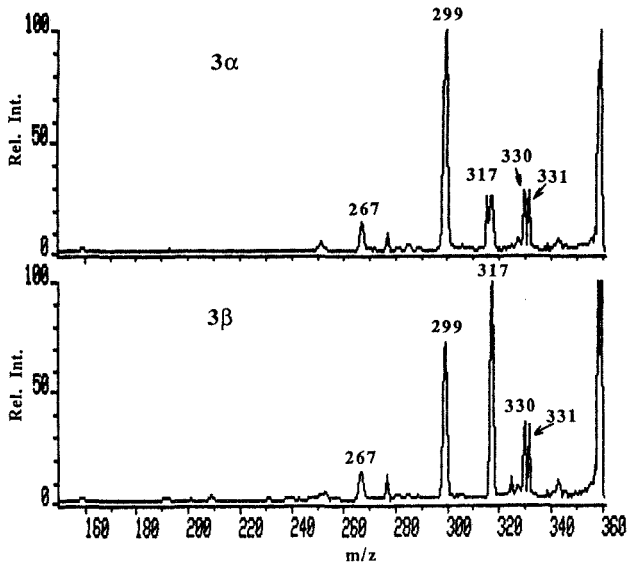


Figure 4. FAB MIKE spectra of the *m/z* 359 ([M + H − $PhCH_2OH$]$^+$) ion of **3α** and **3β**.

The FAB MIKE spectra of the [M + H − $PhCH_2OH$]$^+$ ion (*m/z* 359) of **3α** and **3β** (Fig. 4) show also fragment ions at *m/z* 331 (loss of CO), *m/z* 330 (loss of CHO), *m/z* 317 (loss of C_2H_2O), *m/z* 299 (loss of HCO_2Me) and *m/z* 267 (loss of $PhCH_3$) in different abundances; as can be seen, the base peak for the α-anomer is at *m/z* 299 and for the β-anomer at *m/z* 317.

Hence as the FAB MIKE spectra of the [M + H − $PhCH_2OH$]$^+$ ion of the pairs of anomers are different, this implies that the daughter ions arising from the α- and β-anomers are diastereoisomeric; the fragmentation of the benzyloxy group occurs at the primary position leading to structure *a*. However, this does not exclude at the same time the formation of the daughter ion *b*. This ion at *m/z* 228 is probably a mixture of *a* and *b*.

CONCLUSION

Some saturated and unsaturated *C*-glycosides were studied using the FAB ionization technique. In the FAB mass spectra of the benzylated *C*-glycosides, the expected protonated molecular ions [M + H]$^+$ are not observed as major peaks, the quasimolecular species corresponding to [M + H]$^+$. This phenomenon could give rise to misinterpretation of FAB mass spectra and particularly the determination of the relative molecular masses of unknown samples. For the benzylated *C*-glycosides the anomeric *C*-glycosides showed a distinct ion pattern, and particularly the FAB MIKE and FAB CAD–MIKE spectra of the [M − H]$^+$ ion of the two anomers are different and characteristic of the anomers and could be used as 'fingerprints' of these anomers. The FAB MIKE and FAB CAD–MIKE spectra of the [M − H]$^+$ ion of the benzylated *C*-glycosides also show different fragmentations for the two anomers. These differences could be related to the conformational equilibrium observed for these compounds.

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