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Characterization of the Sulfated Fucose-containing Trisaccharides by Fast Atom Bombardment Tandem Mass Spectrometry in the Study of the Acrosome Reaction-inducing Substance of the Starfish, *Asterias amurensis*

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Fast atom bombardment mass spectrometry (FABMS) and collision-induced dissociation tandem mass spectrometry (CID-MS/MS) were applied in the investigation of the anomeric isomerism of synthetic trisaccharides consisting of xylose, galactose and sulfated fucose {Xyl1 → 3Galα1 → 3(4-OSO_3Na)Fuc} and {Xyl1 → 3Galα1 → 4(3-OSO_3Na)Fuc} and the linkage position of the sulfate group. It was possible to differentiate between various glycosidic linkages in several synthetic trisaccharides. The position of a sulfate group in synthetic methyl *O*-sulfo-α-L-fucopyranoside isomers was elucidated from the fragmentation patterns. Comparing the data from synthetic sulfated trisaccharides with the spectra from the natural compound derived from glycan chains of the acrosome reaction-inducing substance (ARIS) from starfish, the anomeric structure and the position of the sulfate group in the natural sample were determined without ambiguity as Xylβ1 → 3Galα1 → 3(4-OSO_3^-)Fuc, in agreement with the result from an independent study based on nuclear magnetic resonance. © **1998 by John Wiley & Sons, Ltd.**

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KEYWORDS: Fast atom bombardment tandem mass spectrometry; trisaccharide; sulfated fucose; glycosidic linkage; positional isomer

INTRODUCTION

Recently, research on the specific binding during fertilization between sugar chains on the surface of eggs and carbohydrate-recognizing proteins on sperm has been actively carried out and the structures of a variety of glycoconjugates having sulfonic groups have been reported.[1–14] Okinaga *et al.*,[15] using negative fast atom bombardment mass spectrometry (FABMS), reported that a major component of sugar chains in the acrosome reaction-inducing substance (ARIS) of the starfish, *Asterias amurensis*, is the trisaccharide Xyl1 → 3Galα1 → 3(4-OSO_3^-)Fuc or Xyl1 → 3Galα1 → 4(3-OSO_3^-)Fuc. However, fragment ions necessary for determining the complete structure were not present in this FAB mass spectrum because of a low signal-to-noise ratio. To clarify these results, several related saccharides were synthesized and analyzed by FABMS and collision-induced dissociation tandem mass spectrometry (CID-MS/MS) and the relationship between their structures and behaviors in FABMS have been established. These spectra have been compared with those obtained from the sulfated trisaccharide derived from ARIS to determine its anomeric structure and the linkage of the sulfate group, which have been established independently by NMR studies.

FABMS, in particular FAB CID-MS/MS,[16] can be used to differentiate various isomers: structural isomers of sulfomonosaccharides of methyl α-D-galactopyranoside[17] and methyl α-D-mannopyranoside;[18] structural isomers of sulfatides;[19] stereoisomers of aldohexoses;[20] anomeric glycosides;[21] glycosaminoglycan-derived disaccharides;[22] positional isomers of glycosphingolipids and gangliosides;[16] and linkage isomers of acidic trisaccharides Le^x and Le^a.[23]

In this paper, the characterization of anomeric isomers of sulfated trisaccharides and a method for determining the position of the sulfate group in methyl *O*-sulfo-α-L-fucopyranosides using FABMS and CID-MS/MS are described. The compounds studied are shown in Scheme 1. We used five compounds, **1**, **2**, **4**, **5**

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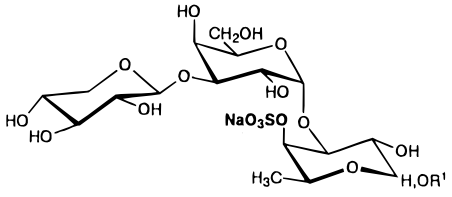
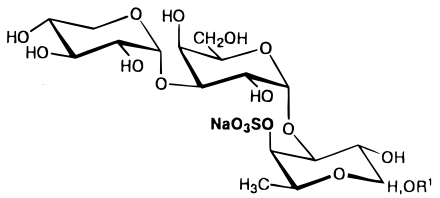
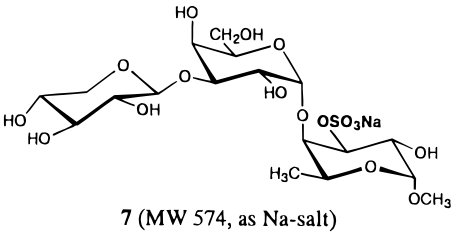


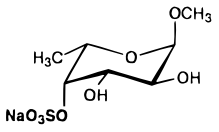
1 R^1=CH_3 (α) (MW 574, as Na-salt)
2 R^1=H (MW 560, as Na-salt)
3 non-sulfated form of **1** (MW 472)



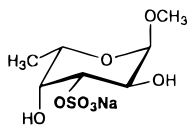
4 R^1=CH_3 (α) (MW 574, as Na-salt)
5 R^1=H (MW 560, as Na-salt)
6 non-sulfated form of **4** (MW 472)



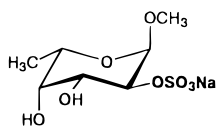
7 (MW 574, as Na-salt)



8 (MW 280, as Na-salt)



9 (MW 280, as Na-salt)



10 (MW 280, as Na-salt)

11 unknown sulfated trisaccharide from ARIS

Scheme 1. Structures of sample compounds.

and **7**, for establishing the anomericity of the linkage between xylose and galactose and/or between galactose and fucose of the sulfated trisaccharide **11**, which is the major glycan fragment derived from ARIS of the starfish, *Asterias amurensis*,[15] with an undetermined structure. Compounds **1**, **2**, **4** and **5** are trisaccharides Xyl1 → 3Galα1 → 3(4-OSO_3Na)Fuc; the reducing terminal residue is a methyl fucoside in compounds **1** and **4**; compounds **2** and **5** were synthesized as the two molecules most likely to be the natural trisaccharide from ARIS, in which the fucose is reducing. Compound **7** is a trisaccharide Xylβ1 → 3Galα1 → 4(3-OSO_3Na)Fuc(OCH_3), which corresponds to the regioisomer of compound **1** in the sulfated fucose moiety (C-3/C-4). The reason why we also used the trisaccharides including the methyl fucoside moiety in addition to the reducing fucose moiety is that the synthetic methylglycosidated analogs **1**, **4** and **7** are effective for elongation of the sugar chain in syntheses in the near future for investigating the structures of the minor glycan fragments derived from ARIS. Because we could not obtain good CID tandem mass spectra of the reducing sulfated monofucoses, we distinguished the position of the sulfate group of the sulfated fucose in the unknown compound **11** by comparing the different cleavage patterns of the bond between galactose and sulfated fucose from the CID tandem mass spectra of trisaccharides **1**, **2**, **4**, **5**, **7** and **11**. It is essential to determine the position of the sulfate group C-3 or C-4 of the sulfated fucose in the unknown sulfated trisaccharide **11** if the anomericity of the linkage between galactose and fucose in compound **11** is determined. Compounds **1**, **2**, **4**, **5** and **7–10** are sodium salts. Compounds **8–10** are linkage regioisomers, i.e. 4-, 3-and 2-sulfates of methyl α-L-fucopyranoside, respectively. They are useful for distinguishing the linkage position of the sulfate group of the sulfated fucose in the synthetic trisaccharide. Trisaccharides **3** and **6** correspond to the non-sulfated analogs of compounds **1** and **4**.

EXPERIMENTAL

Syntheses of compounds **1–10** have been reported elsewhere.[24,25] ARIS, one of the main components of starfish egg coat, is a highly sulfated glycoprotein with a molecular mass of $>10^7$. Glycan chains were obtained from ARIS by solubilizing the egg coat in acidic sea water, followed by ethanol precipitation and pronase digestion. The glycan chains were hydrolyzed with 10 mM sulfuric acid at 100 °C and separated by high-performance anion-exchange chromatography with pulsed amperometric detection (Dionex, USA). Compound **11** was obtained as the major peak and purified.

The FAB mass spectra of the sulfated trisaccharides were recorded using a triple-stage quadrupole mass spectrometer (Finnigan MAT TSQ 700) equipped with a FAB ion source. Glycerol and diethanolamine (DEA) were used as the matrix in the positive- and negative-ion modes, respectively. The FAB mass spectra were recorded under the following conditions: primary beam, xenon; accelerating voltage of the primary ion, 8 kV; collision gas, argon; collision energy, 20 eV; collision gas pressure, 0.1 Pa for monosaccharides and 0.2 Pa for trisaccharides. The data were processed with a DEC Station 2100 computer.

RESULTS AND DISCUSSION

Analysis of the structures of synthetic trisaccharides with different glycosidic linkages

FABMS of the anomeric isomers **1**, **2**, **4**, **5** and **7** resulted in $[M + Na]^+$ and $[M - Na]^-$ ions, corresponding to the sodiated and the desodiated ions in the

positive- and negative-ion mode, respectively. The FAB mass spectra of compound **1** recorded with both polarities are shown in Fig. 1(a) and (b). Figure 1(a) exhibits monomer and dimer peaks from the trisaccharide at *m/z* 597 and 1171, respectively. The peak at *m/z* 303 indicates $[(NaO_3SO)Fuc(OCH_3) + Na]^+$, produced by a hydrogen transfer from the trisaccharide. The peak at *m/z* 115 indicates $[glycerol + Na]^+$. The FAB mass spectra (not shown) of compound **4** are indistinguishable from the spectra of compound **1**. Positive-ion FAB CID tandem mass spectra of $[M + Na]^+$ at *m/z* 597 of compounds **1** and **4** are shown in Fig. 2(a) and (b). Both Fig. 2(a) and (b) show a series of peaks at *m/z* 477, 335, 317, 303, 271, 223 and 143, which correspond to $[M + Na - NaHSO_4]^+$, $[C_2 + H + Na]^+$, $[B_2 - H + Na]^+$, $[Y_1 + H + Na]^+$, $[Y_1 + H + Na - CH_3OH]^+$, $[Y_1 + H + Na - SO_3]^+$ and $[NaHSO_4 + Na]^+$, respectively, as shown in Scheme 2(a). The systematic nomenclature for carbohydrate fragmentations proposed in Ref. 26 is used. The presence of ions which retain the non-reducing terminus, that is, $[C_2 + H + Na]^+$ and $[B_2 - H + Na]^+$, indicates that Na^+ is not localized to the sulfated sugar, but may migrate to the neutral sugar moiety. The C_2, B_2 and Y_1 ions at *m/z* 335, 317 and 303, respectively, support the successful synthesis of the desired structure, in which the fucose at the reducing terminus is sulfated as well as methylglycosidated. The second Y_1-related ion at *m/z* 271 originates from the elimination of methanol from the methoxyl group at fucose C-1, while the third Y_1-related ion at *m/z* 223 is direct evidence that the sulfated group is contained in the methylfucoside moiety. The appearance of a strong peak at *m/z* 465 in Fig. 2(b) corresponds to the cleavage of a xylose in compound **4**,

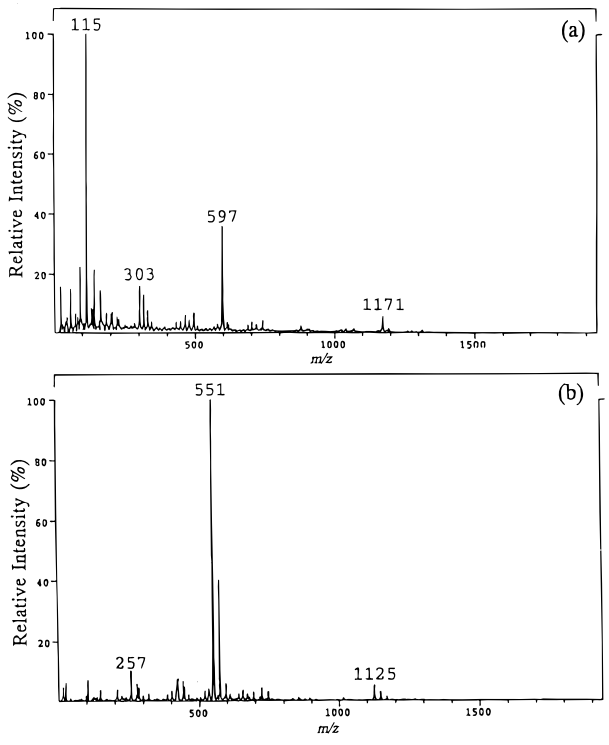


Figure 1. (a) Positive- and (b) negative-ion FAB mass spectra of synthetic sulfated trisaccharides (methyl glycosides); compound **1**. Matrix: (a) glycerol and (b) diethanolamine.

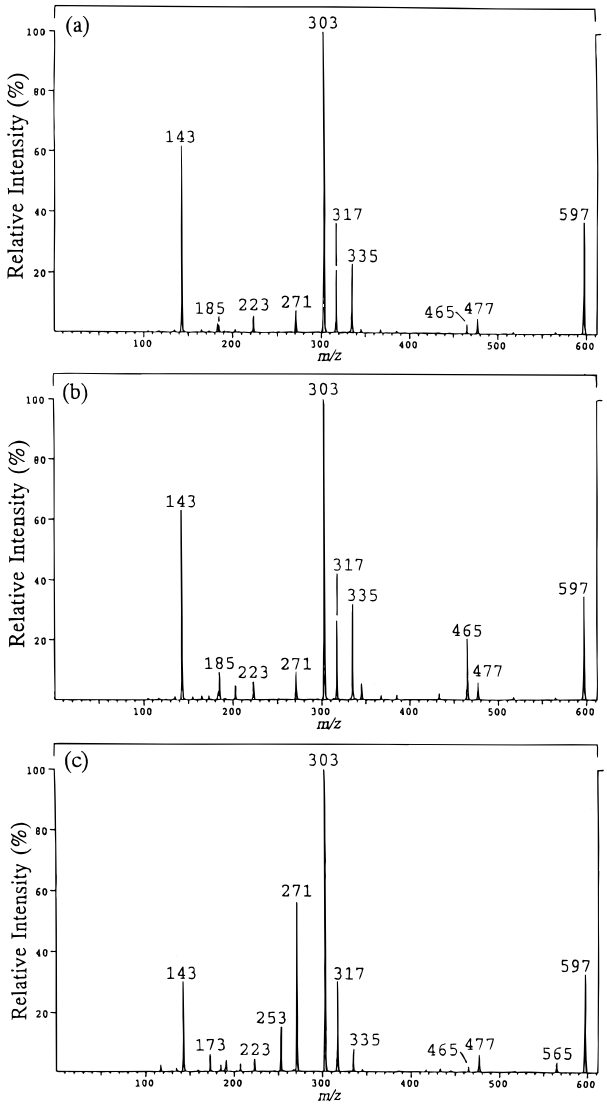
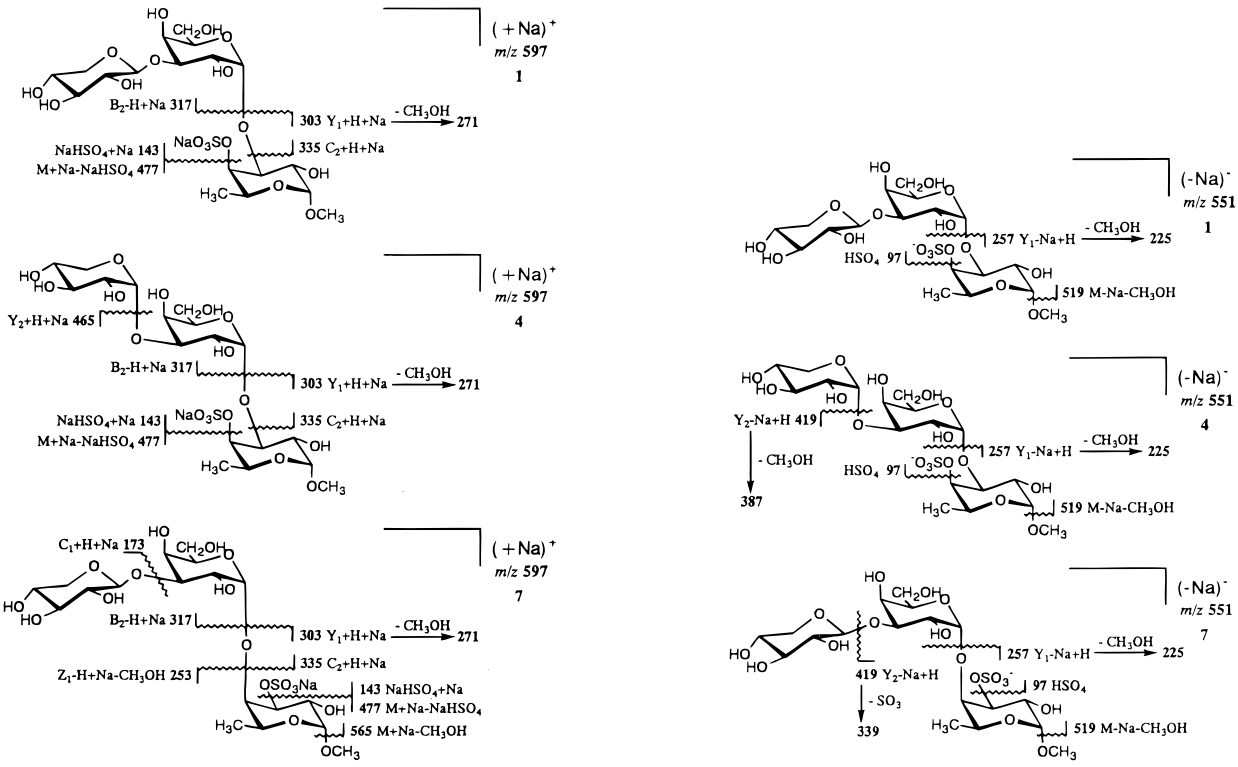


Figure 2. Positive-ion FAB CID tandem mass spectra of synthetic sulfated trisaccharides (methyl glycosides) having $[M + Na]^+$ (*m/z* 597) as the precursor. (a) Compound **1**; (b) **4**; (c) **7**.

$[Y_2 + H + Na]^+$. This should be compared with the weak peak at *m/z* 465 from compound **1** shown in Fig. 2(a). Additionally, the ion with *m/z* 185, corresponding to $[Y_{1/2} + Na]^+$, in Fig. 2(b) appears stronger than that in Fig. 2(a). These differences in CID-MS/MS may be attributed to the difference in the anomeric configuration of xylose, because this is the only structural difference between compounds **1** and **4**. Thus, in this case, it was possible to distinguish the anomeric isomers by FAB CID-MS/MS.

In the negative-ion mode, the FAB mass spectrum of compound **1**, in Fig. 1(b), shows results similar to those obtained in the positive-ion mode, that is, the molecular mass-related ions for the monomer and dimer of the trisaccharide are detected at *m/z* 551 and 1125, respectively. The peak at *m/z* 257 represents $(^-O_3SO)Fuc(OCH_3)$,produced by Y_1 cleavage with a hydrogen transfer from the neutral disaccharide moiety. Compound **4** gives almost the same spectrum (data not shown). Figure 3(a) and (b) show the CID tandem mass



Scheme 2. Fragmentations of compounds **1**, **4** and **7** of (a) positive- and (b) negative-ion CID-MS/MS spectra.

spectra of compounds **1** and **4** having $[M - Na]^-$ (*m/z* 551) as the precursor. Both spectra exhibit ions at *m/z* 519 and 225, due to the loss of methanol from $[M - Na]^-$ and from the ion at *m/z* 257, respectively, as shown in Scheme 2(b). Another ion common to both spectra is observed at *m/z* 97, which corresponds to HSO_4^-.[19] The Y_2 ion at *m/z* 419 in Fig. 3(b) indicates the cleavage of xylose from compound **4**,which is more intense than that from compound **1** shown in Fig. 3(a). The significant intensity of $[Y_2 - CH_3OH]^-$ at *m/z* 387 in the spectrum of compound **4** also supports this. These data suggest that the bond cleavage of the α-glycoside of xylose occurs more easily than the cleavage of the corresponding β-glycoside.

Next, the linkage of galactose and sulfated fucose in compounds **1**, **4** and **7** was considered. Figure 2(c) shows the positive-ion FAB CID tandem mass spectrum of compound **7**. Whereas compounds **1** and **4** yield similar spectra (except for the ion intensity at *m/z* 465, as previously described), differences were seen in the bond cleavage of Galα1 → (NaO_3SO)Fuc(OCH_3) between compound **1** (or **4**) and compound **7** [Scheme 2(a)]. In Fig. 2(c), a new fragmentation corresponding to the Z_1 cleavage with a loss of methanol from $[M + Na]^+$ is observed at *m/z* 253. In the case of compound **7**, the fragment ion at *m/z* 271, corresponding again to the loss of methanol from $[Y_1 + H + Na]^+$, increases this time, while the ion at *m/z* 335 corresponding to the $[C_2 + H + Na]^+$ decreases as compared with compound **1** (or **4**). The appearance of an ion at *m/z* 173, corresponding to $[C_1 + H + Na]^+$, is due to glycosidic cleavage between xylose and galactose on the reducing side of the glycosidic bond. However, compound **1**, which has the same glycosidic bond between xylose and galactose as compound **7**, does not give this ion at *m/z* 173. Hence the unique fragmentations of compound **7** are mainly due to cleavage between the galactose and the sulfated fucose, although the xylosyl–galactose linkage may have some influence over the fragmentation patterns.

The negative-ion FAB CID tandem mass spectra for compounds **1**, **4** and **7** are shown in Fig. 3. The $[Y_1 - Na + H]^-$ at *m/z* 257 is observed to be much more intense [Fig. 3(c)] for compound **7**, which involves the axially oriented glycosidic linkage at the C-4 position of fucose, than for compounds **1** or **4**, which have the equatorially oriented linkage at the C-3 position of fucose [see Fig. 3(a) and (b)]. The ion at *m/z* 519 corresponds to the loss of methanol from $[M - Na]^-$. Ions at *m/z* 419 and 339 represent $[Y_2 - Na + H]^-$ and $[Y_2 - Na + H - SO_3]^-$, respectively [Scheme 2(b)].

Compounds **2** and **5** were synthesized as potential models for the natural compound, which has a free reducing terminus. Otherwise, the remainder of the structures of compounds **2** and **5** are the same as those of compounds **1** and **4**, respectively. The natural compound **11** was assumed from previous studies[15,25] to have a sulfated fucose residue at its reducing terminus. Compounds **2** and **5** showed fragmentation patterns (Figs 4 and 5) similar to those from compounds **1** and **4** on CID-MS/MS except that the former loses water (*m/z* 271) in Scheme 3(a) and *m/z* 225 and 519 in Scheme 3(b) instead of methanol as shown in Scheme 2(a) and (b), respectively. This suggests that it is possible to apply CID-MS/MS to determine the structure of the natural compound **11**, the details of which are described later.

Compounds **3** and **6**, which correspond to non-sulfated forms of compounds **1** and **4**, were studied to see how the sulfate group affects the FAB CID-MS/MS fragmentation of the trisaccharides. Figure 6(a) and (b) show positive-ion CID tandem mass spectra of com-

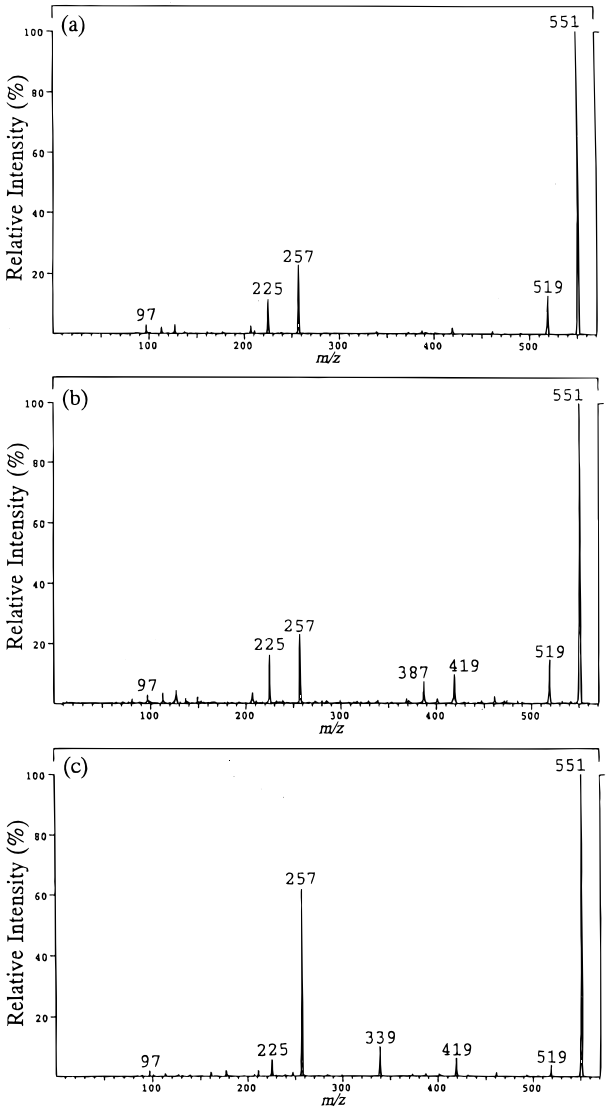


Figure 3. Negative-ion FAB CID tandem mass spectra of synthetic sulfated trisaccharides (methyl glycosides) having $[M - Na]^-$ (*m/z* 551) as the precursor. (a) Compound **1**; (b) **4**; (c) **7**.

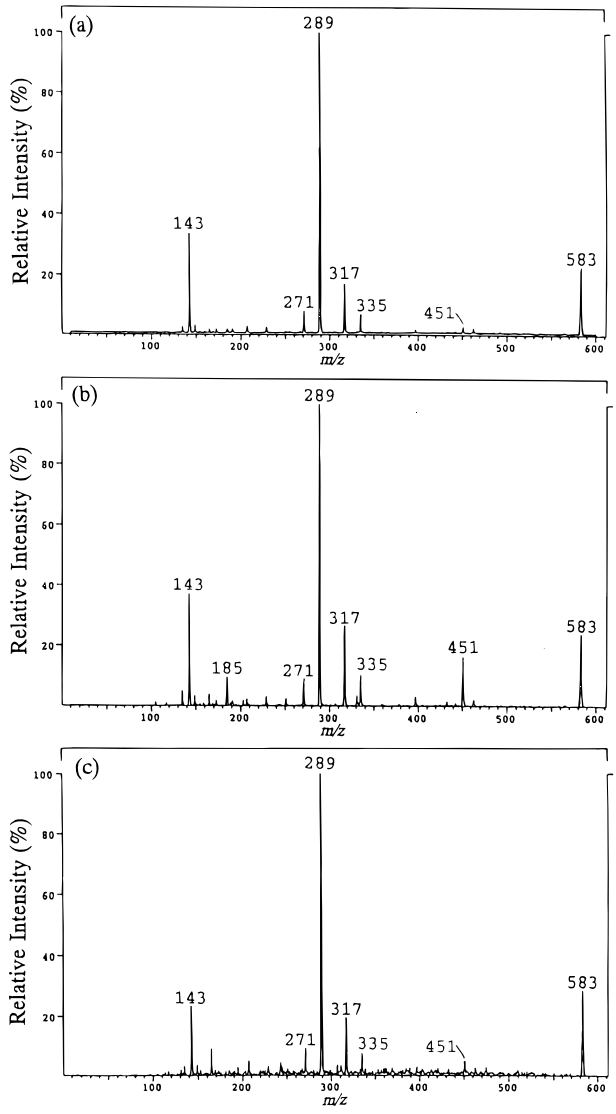


Figure 4. Positive-ion FAB CID tandem mass spectra of synthetic sulfated trisaccharides (free reducing ends) having $[M + Na]^+$ (*m/z* 583) as the precursor. (a) Compound **2**; (b) **5**; (c) **11**.

pounds **3** and **6**, respectively, having $[M + Na]^+$ (*m/z* 495) as the precursor ion. Figure 6(a) shows fragment ions at *m/z* 463, 363, 331, 317, 201, 185, 169 and 155, corresponding to $[M + Na - CH_3OH]^+$, $[Y_2 + H + Na]^+$, $[Y_2 + H + Na - CH_3OH]^+$, $[B_2 - H + Na]^+$, $[Y_1 + H + Na]^+$, $[B_{1/2} + Na]^+$ or $[Y_{1/2} + Na]^+$, $[Y_1 + H + Na - CH_3OH]^+$ and $[B_1 - H + Na]^+$, respectively, as shown in Scheme 4. Compound **6** exhibits ions similar to those from compound **3** as seen in Fig. 6(b) (Scheme 4). However, comparison of these fragment ions in Fig. 6(a) and (b) for compounds **3** and **6** reveals that the ions at *m/z* 363, 331 and 185 for compound **6** are stronger than those from compound **3**. This suggests that the cleavage of xylose from compound **6** occurs more readily than from compound **3**. In other words, α-linked xylose is lost more easily than its β-linked analog, just as in the case of the sulfated analogs **1** *vs.* **4** and **2** *vs.* **5**. When the positive-ion FAB CID tandem mass spectra of compounds **1**, **2**, **4**, **5** and **7**, which contain the sodiosulfate group on the fucose, are compared with those of the non-sulfated analogs **3** and **6**, the most abundant fragment ion for the former group is, as expected, the sodiated ion of sodiosulfated fucose (Y_1 ion). On the other hand, the fragment ion of highest abundance for compounds **3** and **6** is that corresponding to the loss of methyl fucoside [B_2 ion at *m/z* 317, as shown in Figs 6(a) and (b)]. However, the appearance of the $[C_2 + H + Na]^+$ as well as $[B_2 - H + Na]^+$ from the acidic trisaccharides suggests that the positive charge is not localized exclusively on the sulfated fucose but may be attached to neutral sugars as in compounds **1**, **2**, **4**, **5** and **7**. This $[C_2 + H + Na]^+$ ion appearing at *m/z* 335 with significant abundance from compounds **1**, **2**, **4**, **5** and **7** was not observed from the neutral trisaccharides **3** and **6**.

Negative-ion CID tandem mass spectra of compounds **3** and **6** having $[M - H]^-$ at *m/z* 471 as precursor (data not shown) show common fragment ions at *m/z* 339, 177, 149 and 89, which correspond to Y_2^-,

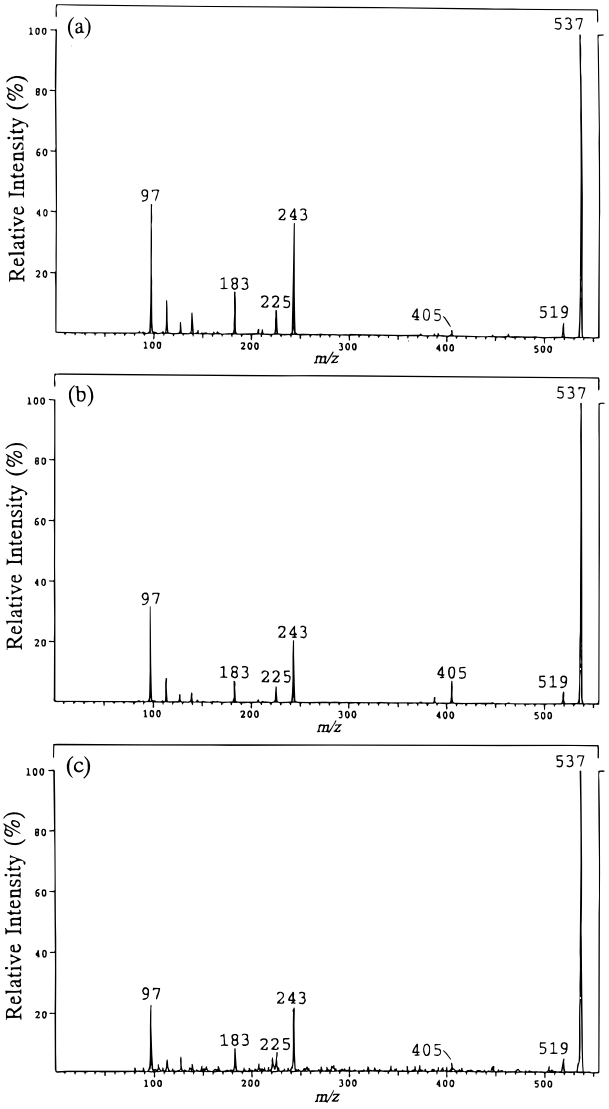


Figure 5. Negative-ion FAB CID tandem mass spectra of sulfated trisaccharides (free reducing ends) having $[M - Na]^-$ (*m/z* 537) as the precursor. (a) Compound **2**; (b) **5**; (c) **11**.

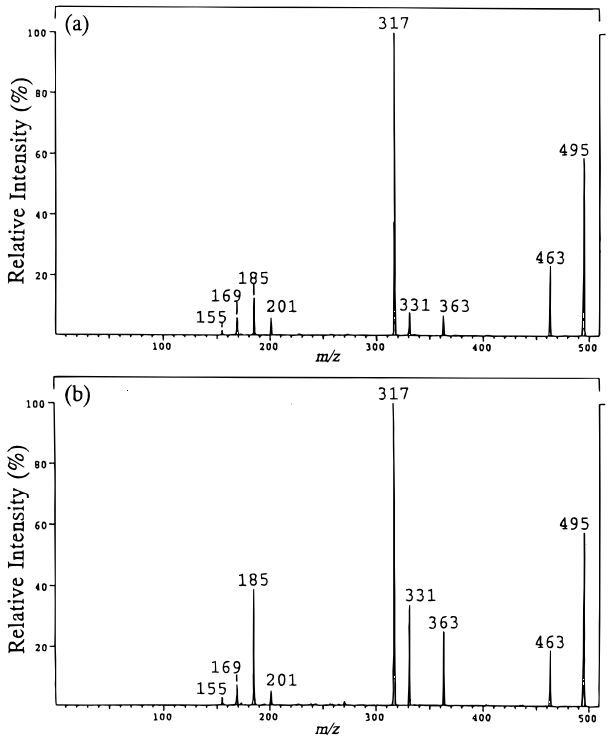
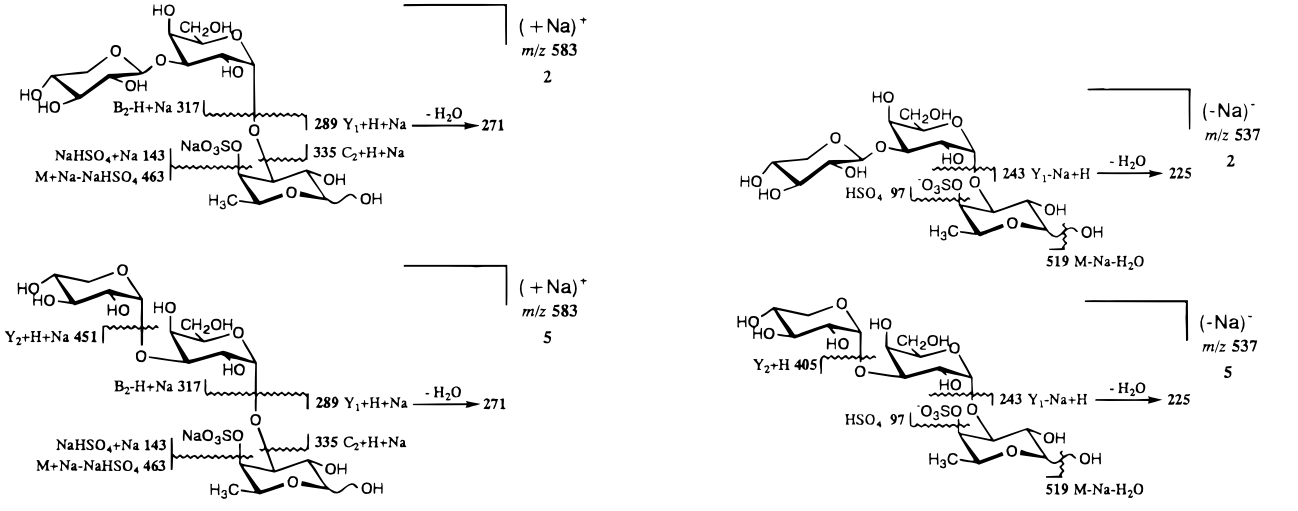


Figure 6. Positive-ion FAB CID tandem mass spectra of synthetic neutral trisaccharides (methyl glycosides) having $[M + Na]^+$ (*m/z* 495) as the precursor. (a) Compound **3**; (b) **6**.

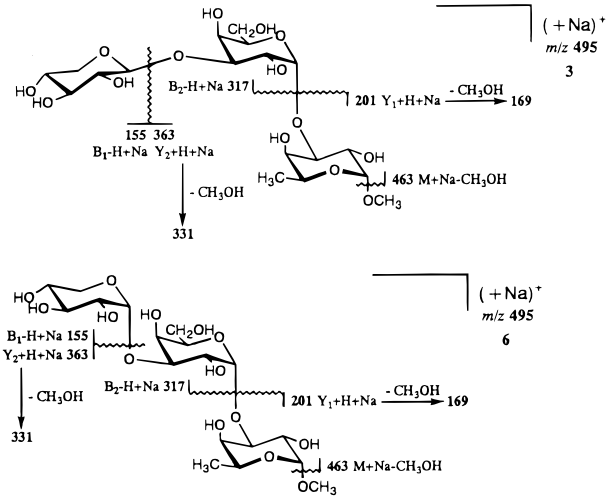
Y_1^-, C_1^- and $[^{0,2}A_1 - H]^-$, respectively. Indeed, the negative-ion FAB CID tandem mass spectra of compounds **3** and **6** show different fragmentation patterns to those of compounds **1** and **4**. Such spectra of compounds **1** and **4** mostly contain ions bearing the desodiated sulfate. Conversely, the negative-ion FAB CID tandem mass spectra of compounds **3** and **6** show some fragment ions which do not contain the fucose moiety. It is clear that the sulfate group has an important role in the negative-ion fragmentation of these structures.

Distinguishing sulfate positional isomers on methyl α-L-fucopyranosides

To determine the position of the sulfate substituent on fucose in the natural trisaccharide, basic knowledge was



Scheme 3. Fragmentations of compounds **2** and **5** of (a) positive- and (b) negative-ion CID-MS/MS spectra.



Scheme 4. Fragmentations of compounds **3** and **6** of positive-ion CID-MS/MS spectra.

obtained by synthesizing three candidate monosaccharides on methyl *O*-sulfo-α-L-fucopyranosides, having the sulfate substituent attached to three different sites (compounds **8**–**10**), and by comparing their CID mass spectra for $[M + Na]^+$ (*m/z* 303) (Fig. 7) and $[M - Na]^-$ (*m/z* 257) (Fig. 8).

In the positive-ion mode, the CID spectra have a prominent ion in common at *m/z* 143, corresponding to $[NaHSO_4 + Na]^+$, and at *m/z* 223, which is produced by the cleavage of SO_3 as $[M + Na - SO_3]^+$ as shown in Scheme 5(a). An ion at *m/z* 117 may correspond to either $^{2,4}X$ (for compounds **8** and **9**) or $^{1,3}X$ (for compounds **9** and **10**) representing a ring-opening ion containing no sodium atom, and another ion at *m/z* 271 may be attributable to the loss of methanol from the $[M + Na]^+$ [Scheme 5(a)]. However, these two ions (*m/z* 117 and 271) together are observed only from compound **9**, in which the sulfate group is linked at C-3 [Fig. 7(b)], while compounds **8** and **10** [Fig. 7(a) and (c)] show neither of these two fragment ions.

In the negative-ion mode, the ion for the loss of methanol (*m/z* 225) is produced from compounds **8** and **9** [Fig. 8(a) and (b)], but not from compound **10**, in which the sulfate group is at C-2 [Fig. 8(c)]. This suggests that the 2-OH is needed for the methanol elimination from $[M - Na]^-$. A ring-opening negative ion at *m/z* 139, corresponding to the $^{2,4}A$ cleavage [Scheme 5(b)], appears with modest abundance from compound **8** [Fig. 8(a)], but is of almost negligible abundance from compound **10** [Fig. 8(c)], in which the sulfate group cannot form a stable six-membered ring with an adjacent OH group. In contrast to the results obtained in the positive-ion mode, the sulfate group gives the ion at *m/z* 97 (HSO_4^-) with almost the same abundance from these compounds rather than serving as an eliminating group. Hence, using both positive- and negative-ion CID-MS/MS, information is obtained on the site of sulfation of the monosaccharide.

The applicability of FAB CID-MS/MS for monosaccharides was examined next for its ability to distinguish the synthetic trisaccharide isomers **1**, **4** and **7**, each having a sulfate substituent at a different position on the methyl α-L-fucopyranoside. A fragment ion $[(NaO_3SO)Fuc(OCH_3) + Na]^+$ (*m/z* 303), rather than

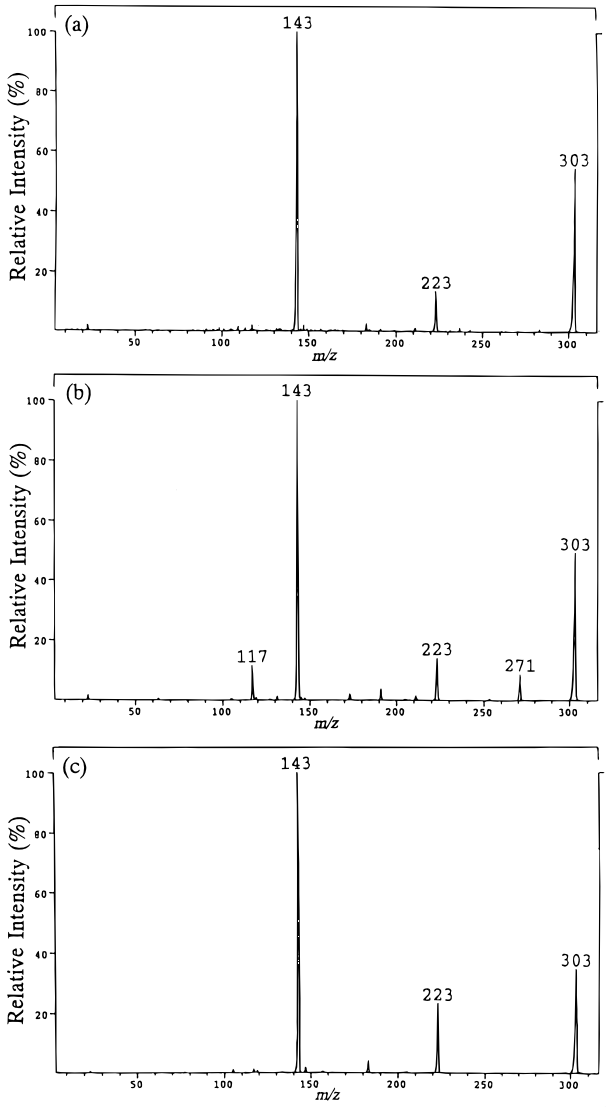


Figure 7. Positive-ion FAB CID tandem mass spectra of sulfated methyl α-L-fucopyranosides having $[M + Na]^+$ (*m/z* 303) as the precursor. (a) Compound **8**; (b) **9**; (c) **10**.

the $[M + Na]^+$ was chosen as the precursor ion for the positive-ion experiment, while $(^-O_3SO)Fuc(OCH_3)$ (*m/z* 257), instead of $[M - Na]^-$,was the precursor ion for the negative product ion spectra for the three trisaccharides.

To accomplish this, the previously described FAB CID tandem mass spectra of isomeric methyl α-L-fucopyranosides with a known sulfate site (compounds **8**, **9** and **10**) were compared with the FAB CID tandem mass spectra of **1**, **4** and **7**, for which the partial structure equivalent to the sulfated methyl fucoside was chosen as the precursor. Through these comparisons we confirmed that FAB CID tandem mass spectra having fragment ions from the trisaccharides as precursor resemble the spectra of the principal fucose analogs and particularly that these spectra reflect the linkage positions of sulfate as evidenced by Figs 7 and 9. That is, compounds **1** and **4** produce product ion spectra (Fig. 9(a) for compound **1** and Fig. 9(b) for compound **4**) that are much like that from compound **8** [Fig. 7(a)], these

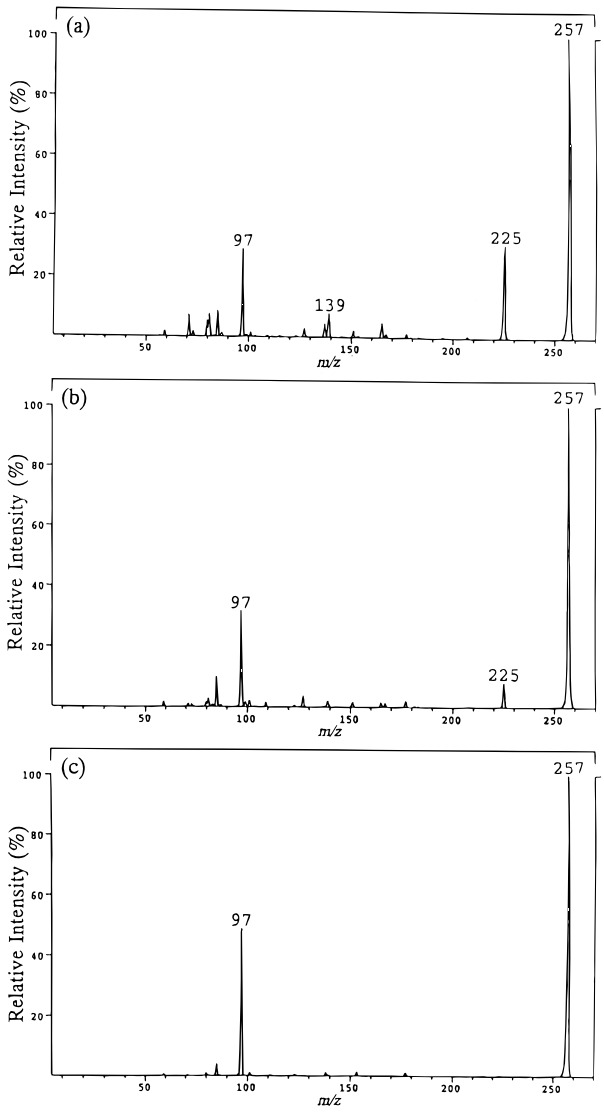


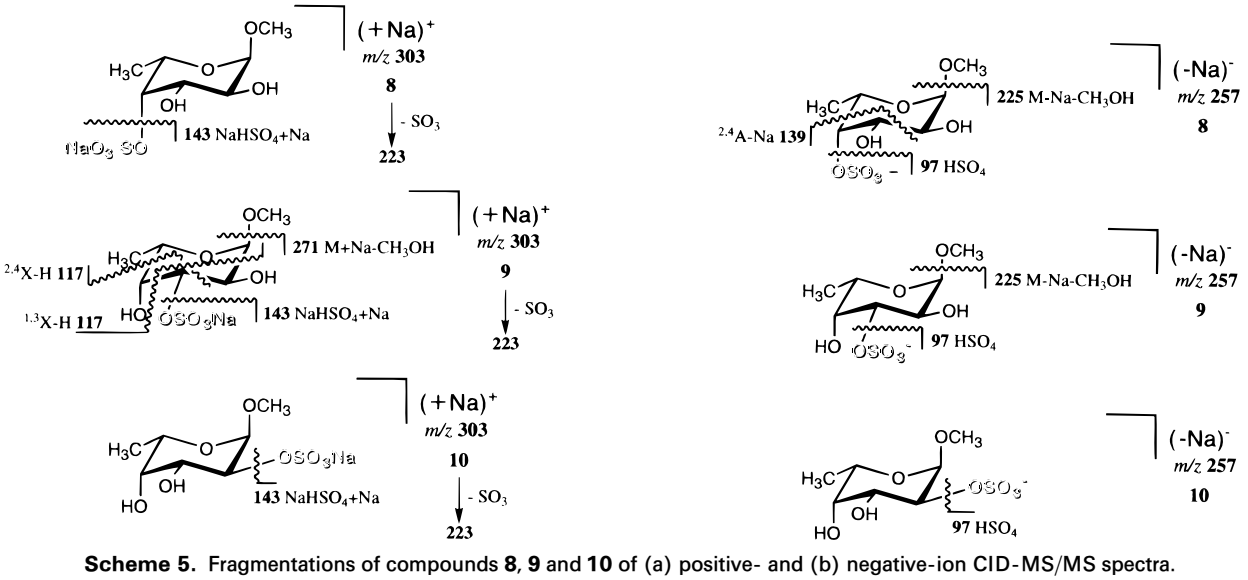
Figure 8. Negative-ion FAB CID tandem mass spectra of sulfated methyl α-L-fucopyranosides having $[M - Na]^-$ (*m/z* 257) as the precursor. (a) Compound **8**; (b) **9**; (c) **10**.

compounds all have the sulfate group located on the 4-OH, while compound **7** yields a CID tandem mass spectrum [Fig. 9(c)] superimposable over that from compound **9** [Fig. 7(b)], both of which compounds have the 3-OH substituted with a sulfate group.

The negative-ion spectra produced similar results (data not shown). This suggests that the sulfated site in an unknown trisaccharide may be determined using FAB CID-MS/MS by choosing the principal partial structure as the precursor ion. On the other hand, the CID tandem mass spectra from compounds **2** and **5**, in which the fucose is reducing, as in the case of the naturally occurring trisaccharide from ARIS, have a different pattern of fragmentation from the spectra of the respective methylglycosidated analogs **1** and **4**. This is probably because the free reducing terminus is in equilibration, yielding a mixture of α- and β-anomers in addition to the open-chain isomer.

Analysis of the structure of the unknown trisaccharide derived from ARIS

The trisaccharide, Xyl1 → 3Gal1 → 3 or 4(4- or 3-OSO_3^-)Fuc, that Okinaga *et al.*[15] reported is the major glycan structure in the ARIS of *Asterias amurensis*. As described above, trisaccharides **2** and **5** were synthesized as potential candidates for the natural trisaccharide from ARIS, but the linkage position of the sulfate in the natural trisaccharide was unknown. The structurally unknown natural trisaccharide **11** was analyzed first using FABMS in the normal scanning mode. Figure 10(a) and (b) show the positive- and negative-ion FAB mass spectra, in which pseudomolecular ions were detected at *m/z* 583 and 537, corresponding to $[M + Na]^+$ and $[M - Na]^-$, respectively, where M is equivalent to Xyl → Gal → (NaO_3SO)Fuc. Hence the molecular mass of compound **11** was confirmed to be consistent with the structure determined by NMR.[25] Second, comparison of the FAB CID tandem mass specrta of $[M + Na]^+$ and $[M - Na]^-$ ions of **11** [Figs



Scheme 5. Fragmentations of compounds **8**, **9** and **10** of (a) positive- and (b) negative-ion CID-MS/MS spectra.

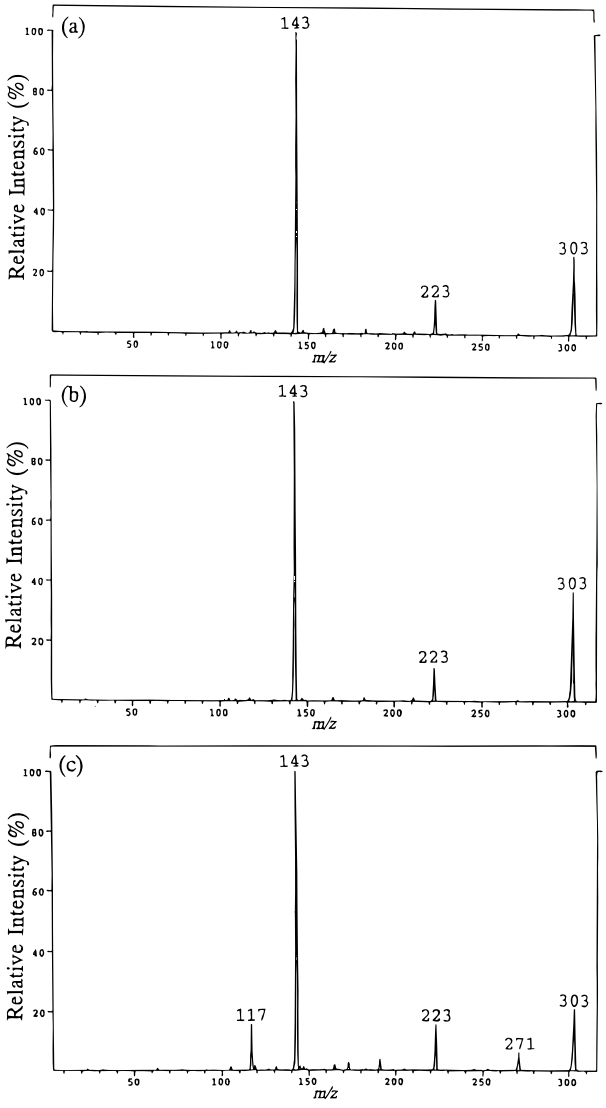


Figure 9. Positive-ion FAB CID tandem mass spectra of synthetic sulfated trisaccharides (methyl glycosides) having a fragment ion corresponding to *m/z* 303 as the precursor. (a) Compound **1**; (b) **4**; (c) **7**.

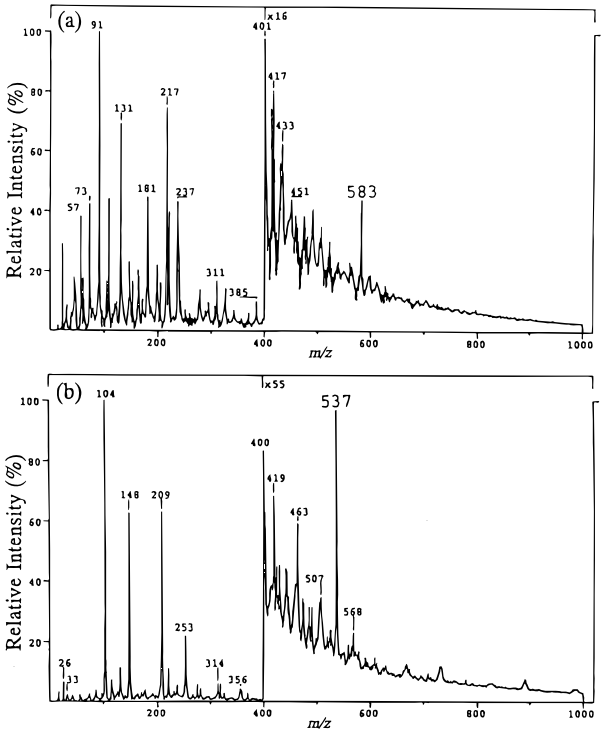


Figure 10. (a) Positive- and (b) negative-ion FAB mass spectra of the ARIS-derived sulfated trisaccharide (free reducing end); compound **11**.

4(c) and 5(c), respectively] with the corresponding spectra of the known trisaccharide **2** and **5** was carried out, to determine the configuration of xylose. It was found that the CID spectra from compound **11** are most comparable to those from compound **2** by looking at the relative abundances of the fragment ions at *m/z* 451 and 185 in Fig. 4 in the positive-ion mode and *m/z* 405 in Fig. 5 in the negative-ion mode. Thus it is indicated that compound **11** has a β-xylopyranosyl linkage, which supports the results of an independent study using NMR. Next, to determine the position of the sulfate group, a comparison of the CID tandem mass spectra of the sulfated fucose moieties in compounds **2**, **5** and **11** was made as suggested earlier in this paper. Unfortunately, however, clear partial CID tandem mass spectra for the trisaccharides **2**, **5** or **11**, all of which have a free reducing terminus, could not be obtained when the partial structure representing the sulfated fucose was chosen as the precursor, because the ion abundances for the positive and negative precursors were too weak. Nevertheless, the position of the sulfate group in **11** (i.e. the position of the sulfate group on fucose) was identified as *O3*-glycosyl-*O4*-sulfofucose based on the fact that the FAB CID tandem mass spectra of **11** in both the positive- and negative-ion modes are indistinguishable from those from compound **2**, but different from those from **5**, when $[M + Na]^+$ and $[M - Na]^-$ were chosen as the precursor ions.Therefore, it was shown by comparing the FAB CID tandem mass spectra of synthetic compounds **2** and **5** with the natural compound **11** that the structure of the sulfated trisaccharide from the ARIS of the starfish, *Asterias amurensis*, is Xylβ1 → 3Galα1 → 3(4-OSO_3^-)Fuc.

CONCLUSION

FAB CID-MS/MS of sulfated trisaccharides has the possibility of providing significant information about their structures, e.g. the sequence of three sugars, linkages between adjacent sugars and the position of a sulfate group. The α-glycosidic linkage between xylose and galactose resulted in higher ion abundances in both the positive- and negative-ion modes. We assume that the reduced nucleophilicity of the ring oxygen in the β-xylopyranose due to hydrogen bonding with the *O4*-hydroxyl group in the galactose makes the glycosidic cleavage of xylose C1—O less facile, while such hydrogen bonding is not likely to occur in the case of the α-anomer, making the axially oriented lone pair of the ring oxygen available to the xylose C1—O cleavage. The apparent tendency for easier cleavage of the α-

glycosidic bond than the β-anomer's is thus explicable. Regarding the positions of the sulfate group on the methyl α-L-fucopyranosides, the $^{1,3}X$ or $^{2,4}X$ ion at m/z 117 was observed together with another at m/z 271, corresponding to the loss of methanol from $[(NaO_3SO)Fuc(OCH_3) + Na]^+$,specifically for the C-3 sulfated methyl fucoside in the positive-ion FAB CID tandem mass spectra. On the other hand, negative-ion FAB CID-MS/MS of $[M - Na]^-$ for three sulfated methyl fucosides yields an ion at m/z 225 corresponding to the loss of methanol from $(^-O_3SO)Fuc(OCH_3)$ (m/z 257) for those in which the 2-OH is available to form a stable five-membered ring with the methoxyl group.

FAB CID-MS/MS was applicable to trisaccharides **2** and **5** and extended to the unknown natural trisaccharide **11**, where a partial structure corresponding to the principal sulfated methyl fucoside was chosen as the precursor. It is clear that fragmentation of the natural trisaccharide **11** is most similar to that of compound **2**,and not to **5**. Therefore, it suggests that the natural compound **11** from the ARIS of the starfish, *Asterias amurensis*, consists of Xylβ1 → 3Galα1 → 3(4-OSO_3^-)Fuc. By synthesizing several compounds analogous to possible forms of the natural compound and then comparing the FAB CID tandem mass spectra from those known compounds with those from the natural unknown compound, the structure of an infinitesimal amount of a natural compound, which would otherwise be difficult to analyze because too little sample is available for ^{13}C NMR, can be identified.

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REFERENCES

1. G. K. SeGall and W. J. Lennarz, *Dev. Biol.* **71**, 33 (1979).
2. G. K. SeGall and W. J. Lennarz, *Dev. Biol.* **86**, 87 (1981).
3. E. Topfer-Petersen, A. E. Friess, H. Nguyen and W. B. Schill, *Histochemistry* **83**, 139 (1985).
4. T. Matsui, I. Nishiyama, A. Hino and M. Hoshi, *Dev. Growth Differ.* **28**, 339 (1986).
5. R. DeSantis and R. Pinto, *Dev. Growth Differ.* **29**, 617 (1987).
6. M. G. O'Rand, E. E. Widgren and S. Fisher, *Dev. Biol.* **129**, 231 (1988).
7. M. Abdullah and A. L. Kierszenbaum, *J. Cell Biol.* **108**, 367 (1989).
8. D. R. P. Tulsiani, M. D. Skudlarek and M.-C. Orgebin-Crist, *J. Cell Biol.* **109**, 1257 (1989).
9. D. J. Miller and R. L. Ax, *Mol. Reprod. Dev.* **26**, 184 (1990).
10. P. L. DeAngelis and C. G. Glabe, *Biochim. Biophys. Acta* **1037**, 100 (1990).
11. P. M. Wassarman, *Develop.* **108**, 1 (1990).
12. E. Mori, S. Takasaki, J. L. Hedrick, N. J. Wardrip, T. Mori and A. Kobata, *Biochemistry* **30**, 2078 (1991).
13. S. Noguchi, Y. Hatanaka, T. Tobita and M. Nakano, *Eur. J. Biochem.* **204**, 1089 (1992).
14. D. J. Miller, M. B. Macek and B. D. Shur, *Nature (London)* **357**, 589 (1992).
15. T. Okinaga, Y. Ohashi and M. Hoshi, *Biochem. Biophys. Res. Commun.* **186**, 405 (1992).
16. B. Domon and C. E. Costello, *Biochemistry* **27**, 1534 (1988).
17. W. Heerma, C. Versluis, W. Kulik, R. R. Contreras and J. P. Kamerling, *Biomed. Environ. Mass Spectrom.* **17**, 257 (1988).
18. L. Tip, W. Heerma, R. R. Contreras and J. P. Kamerling, *Biol. Mass Spectrom.* **20**, 94 (1991).
19. Y. Ohashi and Y. Nagai, *Carbohydr. Res.* **221**, 235 (1991).
20. J. W. Dallinga and W. Heerma, *Biomed. Environ. Mass Spectrom.* **18**, 363 (1989).
21. M. Brakta, B. Chaguir, D. Sinou, J. Banoub and M. Becchi, *Org. Mass Spectrom.* **27**, 331 (1992).
22. D. J. Lamb, H. M. Wang, L. M. Mallis and R. J. Linhardt, *J. Am. Soc. Mass Spectrom.* **3**, 797 (1992).
23. T. Ii, Y. Ohashi, T. Ogawa and Y. Nagai, *Glycoconj. J.* **13**, 273 (1996).
24. I. G. Leder, *J. Carbohydr. Chem.* **12**, 95 (1993).
25. K. Hiruma and H. Hashimoto, *J. Carbohydr. Chem.* **14**, 879 (1995).
26. B. Domon and C. E. Costello, *Glycoconj. J.* **5**, 397 (1988).