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Differentiation Among the Four Diastereomers of Benzyloxycarbonyl-protected γ-Hydroxyornithine in Negative-ion Fast Atom Bombardment Mass Spectrometry

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Discrimination among the four γ-hydroxyornithine diastereomers was studied by fast atom bombardment mass spectrometry (FABMS). It is impossible to distinguish among the four diastereomers of this amino acid by positive- and negative-ion FAB and collisionally activated dissociation MS, but benzyloxycarbonyl group protection of the α- and δ-amino groups in γ-hydroxyornithine allows differentiation among the diastereomers in negative-ion FABMS. The negative-ion mass spectra of benzyloxycarbonyl-protected γ-hydroxyornithine diastereomers showed differences among the abundances of the molecule ion $[M - H]^-$, the dehydrated ion $[M - H - H_2O]^-$ due to the loss of the γ-hydroxyl group and the fragment ions formed from both $[M - H]^-$ and $[M - H - H_2O]^-$ ions. On the other hand, no difference was found between the fragmentations of the benzyloxycarbonyl-protected *enantiomers* of ornithine in negative-ion FABMS. These results indicate that the orientation of the γ-hydroxyl group and the existence of two benzene rings in the benzyloxycarbonyl group are important factors which are responsible for the fragmentations of the four benzyloxycarbonyl-protected γ-hydroxyornithine diastereomers in negative-ion FABMS. These studies also showed that the negative-ion FABMS for benzyloxycarbonyl-protected γ-hydroxyornithine diastereomers is a useful method for determining the configuration of each diastereomer of γ-hydroxyornithine.

INTRODUCTION

It has generally been assumed until a few years ago that it was too difficult to discriminate between chiral compounds by mass spectrometry. However, several recent reports have been made concerning the differentiation of various steric isomers by mass spectrometry. Leprevote *et al.*[1] reported the stereochemical differentiation of indole alkaloids and Hofmeister and Leary[2] also reported the chiral recognition of lithium-coordinated diols by fast atom bombardment mass spectrometry (FABMS) in combination with tandem MS. Sawata *et al.*[3] reported the enantioselectivity of a modified carbohydrate derivative towards enantiomeric alkylammonium ions by FABMS. Selva *et al.*[4] showed the differentiation of diastereomeric aminotetralines by the metastable ion spectra of 2-oxazolidinone derivatives on electron impact ionization. We have also previously reported that significant differences were observed in negative-ion FABMS among the abundances of the fragment ions formed by the cleavage of the benzyloxycarbonyl (Z) group from the Z-protected tri and tetrapeptides containing L-proline (Pro), depending on the numbers and positions of the prolyl residues.[5–7] Our results imply that the cleavage of the Z-group moiety reflects the conformational difference of the peptide derivatives due to the existence of Pro. Therefore, we applied this observation to discrimination between *erythro* and *threo* forms of γ-hydroxy-L-ornithine (γ-Hy-L-Orn) and briefly reported that negative-ion FABMS for Z-protected γ-Hy-L-Orn diastereomers was a useful method for discriminating between these two diastereomers.[8]

γ-Hydroxyornithine (γ-HyOrn) is a diamino monocarboxylic acid which, by virtue of its two asymmetric centres, may exist as four optically active stereoisomers, *erythro* and *threo* forms of γ-Hy-L-Orn and those of γ-Hy-D-Orn (Fig. 1). γ-Hy-L-Orn diastereomers have reportedly been found in plants.[9] However, the physiological role of this amino acid in plants has not yet been elucidated. In studies on the stereochemistry of γ-

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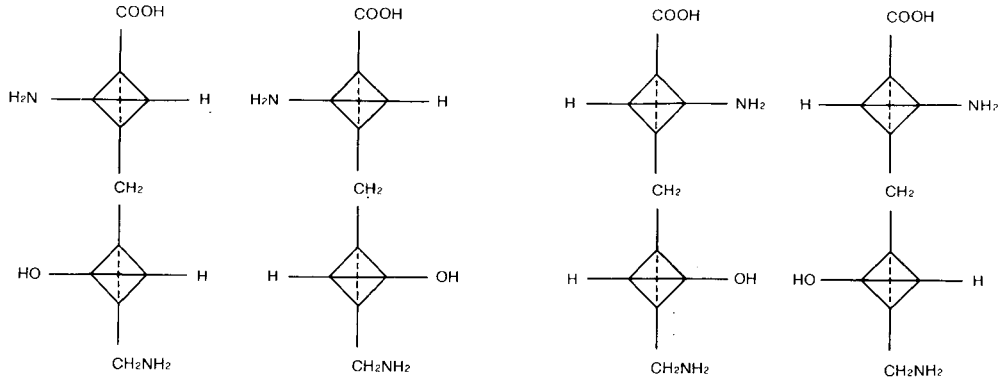

erythro-γ-Hy-L-Orn *threo*-γ-Hy-L-Orn *erythro*-γ-Hy-D-Orn *threo*-γ-Hy-D-Orn

Figure 1. Fisher projections of four stereoisomers of γ-hydroxyornithine.

HyOrn, it would be very important to distinguish between the L- and D-forms of the diastereomeric compounds. We therefore prepared the two diastereomers of γ-Hy-D-Orn and examined the difference in the fragmentations among the four Z-protected γ-HyOrn diastereomers in FABMS. In addition, to examine the effect of the γ-hydroxyl group in γ-HyOrn on the fragmentations of these compounds, we also examined the difference in the fragmentations between L- and D-enantiomers of Z-protected Orn and Lys. As a result, it was impossible to discriminate between the enantiomers of these two amino acids by FAB and collisionally activated dissociation (CAD) MS.

In this paper, we report that the orientational difference of the γ-hydroxyl group and the existence of the two benzene rings in the four Z-protected γ-HyOrn diastereomers are important factors which are responsible for the fragmentations of these compounds in negative-ion FABMS and, therefore, negative-ion FABMS for Z-protected γ-HyOrn is considered to be a useful method for differentiating among the four γ-HyOrn diastereomers.

EXPERIMENTAL

Materials

L-Orn hydrochloride (HCl) was obtained from Wako Pure Chemical Industries (Osaka, Japan) and D-Orn HCl from Kokusan Chemical (Tokyo, Japan). Both L- and D-Lys HCl were purchased from the Protein Research Foundation (Osaka, Japan). γ-HyOrn was prepared and the four racemic diastereomers of this amino acid were separated by the method of Mizusaki *et al.*[10] *N,N'*-Z-protection of the four γ-HyOrn diastereomers and the enantiomers of Orn and Lys was performed by using benzyloxycarbonyl chloride. The purity of each compound was checked by thin-layer chromatography on Kieselgel 60 PF_{254} plates (Merck, Darmstadt, Germany) with chloroform–methanol–ethyl acetate (4:3:1, v/v/v) and butan-1-ol–acetic acid–water (4:1:1, v/v/v) as developing systems. The chemical structure of each compound was determined with the aid of field desorption and FAB mass spectrometry. All other chemicals were of analytical or reagent grade.

Measurement conditions for FABMS

All mass spectra were acquired with a JEOL (Tokyo, Japan) SX/SX102 tandem mass spectrometer of BEBE geometry, which was controlled by a JEOL DA-7000 data system. Positive- and negative-ion FAB mass spectra were obtained by using only the first spectrometer. The samples were diluted in dimethylformamide at a concentration of 0.1 μg μl^{-1}. The solution (0.5 μl) was then mixed with glycerol (0.5 μl) and subjected to analysis. To examine the effect of the matrix, triethanolamine, *m*-nitrobenzyl alcohol and triethylene glycol[8] were used, which possess a higher affinity for protons, greater hydrophobicity and higher polarity than glycerol, respectively. As a consequence, it was impossible to discriminate among the four Z-protected γ-HyOrn diastereomers in both positive- and negative-ion modes when matrices other than glycerol were used. Therefore, glycerol was used as a matrix for the differentiation among the four Z-protected γ-HyOrn diastereomers. The ions were produced by bombardment with a neutral xenon atom at 5 kV. The mass range (*m/z* 1–1000) was scanned for 5 s under an ion source accelerating potential of 10 kV, and the averaged peak intensities were recorded in decade scans. The pseudo-molecule ions generated by FABMS were selected as precursor ions, and then collided with argon molecules in the third field-free region. The argon pressure was sufficient to attenuate the primary ion beam by 50%. The fragment ions were dispersed by the second spectrometer and the spectra were recorded as CAD spectra.

RESULTS AND DISCUSSION

FABMS of γ-HyOrn diastereomers

In the positive-ion mass spectrum of *erythro*-γ-Hy-L-Orn, a protonated molecule ion peak at *m/z* 149 is the base peak, and peaks of an ion at *m/z* 75, which is assigned as $[CH_2CH(OH)CH_2NH_2 + H]^+$ (side-chain),

and an ion at *m/z* 45, assigned as carboxylic acid, were observed with fairly strong intensity. The abundance of a fragment ion at *m/z* 131, which is due to the loss of H_2O, was very weak. In the FAB (CAD) tandem mass spectrum of the $[M + H]^+$ ion for this compound, the dehydrated ion at *m/z* 131 was observed with strong abundance whereas the abundances of the other fragment ions were very weak. Similar cleavage patterns were also obtained in the positive-ion and CAD mass spectra for the other three γ-HyOrn diastereomers.

In the negative-ion mass spectrum of *erythro*-γ-Hy-L-Orn, a molecule ion peak at *m/z* 147 is the base peak, and an ion at *m/z* 59, which is assigned as $[CH(OH)CH_2NH_2 - H]^-$, was observed. In the FAB (CAD) tandem mass spectra of the $[M - H]^-$ ion for this compound, the dehydrated ion at *m/z* 129 from the $[M - H]^-$ ion was observed with strong abundance without main fragment ions. Analogous cleavage patterns were obtained for the other three γ-HyOrn diastereomers. These results indicate that the conformational difference among the four γ-HyOrn diastereomers has no effect on their fragmentations in positive- and negative-ion FAB and CAD mass spectrometry. It is therefore impossible to discriminate among the four γ-HyOrn diastereomers without a modification thereof by FABMS.

FABMS for Z-protected γ-HyOrn diastereomers

Positive-ion FABMS. In the positive-ion mass spectrum of *erythro*-Z-γ-Hy-L-Orn(Z), a molecule ion $[M + H]^+$ at *m/z* 417 was the base peak and the dehydrated ion at *m/z* 399 was observed with fairly strong abundance. On the other hand, in the mass spectra of *threo*-Z-γ-Hy-L-Orn(Z) and both *erythro*- and *threo*-Z-γ-Hy-D-Orn(Z), the dehydrated ion was the base peak, and the $[M + H]^+$ ion was almost not detected. In addition, no significant difference was found among the cleavage patterns of the main fragment ions, for example, $[399 - CO_2H]^-$ at *m/z* 355, $[M - C_6H_5CH_2O]^-$ at *m/z* 307 and $[399 - C_6H_5CH_2OCO]^-$ at *m/z* 265, for the three diastereomers. Therefore, *erythro*-Z-γ-Hy-L-Orn(Z) can be distinguished from the other three diastereomers, but it is impossible to distinguish among the three diastereomers except for *erythro*-Z-γ-Hy-L-Orn(Z) by positive-ion FABMS. In the CAD spectra of the $[M + H]^+$ ion for the four Z-protected γ-HyOrn diastereomers, a fragment ion at *m/z* 399, which is due to the loss of the γ-hydroxyl group from the $[M + H]^+$ ion, and an ion at *m/z* 373, which is assigned as $[M + H - CO_2H + H]^+$, were observed with strong abundances and similar cleavage patterns were observed among them. It is therefore impossible to discriminate among the four γ-HyOrn diastereomers from the positive-ion CAD mass spectra.

Negative-ion FABMS. As the solubility of the Z-protected γ-Hy-D-Orn diastereomers in glycerol was lower than that of the corresponding γ-Hy-L-Orn diastereomers, the cleavage patterns of these compounds were compared at the same sample concentrations. Figure 2(a)–(d) shows the negative-ion FAB mass spectra for the four Z-protected γ-HyOrn diastereomers. In these spectra, the cleavage patterns were observed when an ion at *m/z* 275, which is assigned to $[\text{glycerol} \times 3 - H]^-$, was the base peak. In the spectrum of *erythro*-Z-γ-Hy-L-Orn(Z), a molecule ion $[M - H]^-$ at *m/z* 415 was observed with strong abundance, but the dehydrated ion $[M - H - H_2O]^-$ at *m/z* 397 only had very weak abundance. The $[M - C_6H_5CH_2O - 2H]^-$ ion at *m/z* 307 and the $[M - 135]^-$ ion at *m/z* 281 were also observed as fairly abundant fragment ions formed by the cleavage of the Z group from the $[M - H]^-$ ion. On the other hand, in the spectrum of *threo*-Z-γ-Hy-L-Orn(Z), the abundance of the dehydrated ion was very strong, whereas that of the molecule ion was very weak. The fragment ion $[M - 109]^-$ at *m/z* 307, the ion $[397 - C_6H_5CH_2O - H]^-$ at *m/z* 289 and the ion $[M - H_2O - 135]^-$ at *m/z* 263 were observed. The abundances of the fragment ions at *m/z* 307 and 281 formed by the cleavage of the Z group from the $[M - H]^-$ ion were strong for the *erythro* form, whereas those at *m/z* 289 and 263 from the dehydrated ion were strong for the *threo* form. These results indicate that elimination of the γ-hydroxyl group from the Z-protected *threo* form was easier than that from the corresponding *erythro* form under the FABMS conditions. We have reported previously that stereoselective elimination of the γ-hydroxyl group was observed between *erythro*- and *threo*-γ-Hy-L-Orn and the abundance of the dehydrated ion for the *erythro* form was very weak.[8] However, when the mass spectrum was obtained at a lower concentration of *erythro*- and *threo*-Z-γ-Hy-L-Orn(Z), the abundance of the dehydrated ion for the *erythro* form increased only slightly, although no change was observed regarding this ion for the *threo* form. It is therefore likely that the formation of the dehydrated ion depends on the concentration of *erythro*-Z-γ-Hy-L-Orn(Z).

In the mass spectra of the Z-γ-Hy-D-Orn(Z) diastereomers, the abundance of the dehydrated ion was much stronger than that of the $[M - H]^-$ ion for the *erythro* form, whereas that of the former was slightly weaker than that of the latter for the *threo* form. Similar fragment ions were observed for the Z-protected D-forms of γ-HyOrn as well as for the L-forms. However, a significant difference was found between the abundance of the fragment ion at *m/z* 281 for the *threo* form and that for the *erythro* form in Z-γ-Hy-D-Orn. The cleavage patterns for *erythro*-Z-γ-Hy-L-Orn(Z) were similar to those for *threo*-Z-γ-Hy-D-Orn(Z), whereas those for *threo*-Z-γ-Hy-L-Orn(Z) were also similar to those for *erythro*-Z-γ-Hy-D-Orn(Z). The dehydrated ion was not detected in the negative-ion mass spectra for the four γ-HyOrn diastereomers. It was also not observed in the mass spectra for Z-protected L- and D-Orn, suggesting that the hydroxyl group was not lost from the α-carboxylic acid in the diastereomers. These data showed that the elimination of the γ-hydroxyl group from both Z-protected *threo*-γ-Hy-L-Orn and *erythro*-γ-Hy-D-Orn was more affected than those from both Z-protected *erythro*-γ-Hy-L-Orn and *threo*-γ-Hy-D-Orn owing to the Z-group protection of both the α- and δ-amino groups in γ-HyOrn. The cleavage patterns for Z-L-Orn(Z) were almost the same as those for Z-D-Orn(Z) in the FAB and CAD mass spectra. Similar results were obtained for the mass spectra for the enantiomers of Z-protected Lys.

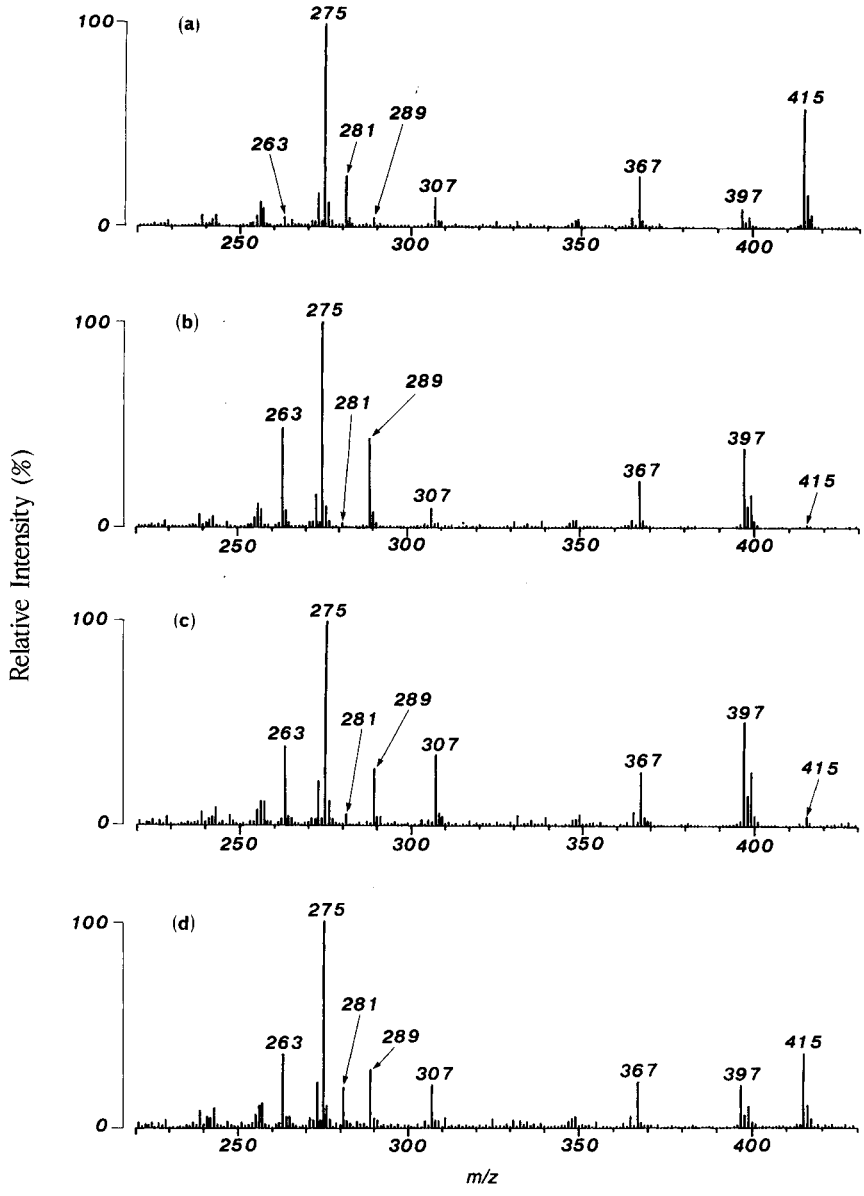

Figure 2. Negative-ion FAB mass spectra for four Z-protected γ-HyOrn diastereomers: (a) *erythro*-Z-γ-Hy-L-Orn(Z); (b) *threo*-Z-γ-Hy-L-Orn(Z); (c) *erythro*-Z-γ-Hy-D-Orn(Z); (d) *threo* Z-γ-Hy-D-Orn(Z). Glycerol was used as a matrix. The ion at *m/z* 367 is assigned as $[\text{glycerol} \times 4 - H]^-$.

Therefore, the differences in the cleavage patterns among the four γ-HyOrn diastereomers may be due to the existence of the γ-hydroxyl group and the two benzene rings in these derivatives. It is likely that the difference in the fragmentations of these diastereomers may depend on the solvation of each diastereomer by glycerol, which is due to the interaction through the hydrogen bonding between each diastereomer and glycerol. These results imply that the orientational difference of the γ-hydroxyl group among the diastereomers is the primary factor responsible for the fragmentations in the negative-ion FABMS conditions. Table 1 shows the relative intensities of the peaks of the molecule ion and the main fragment ions in the negative-ion FAB mass spectra of the four Z-protected γ-HyOrn diastereomers when the ion $[\text{glycerol} \times 3 - H]^-$ at *m/z* 275 was the base peak. Further, the intensities are given in parentheses when the ion with the strongest intensity, which is formed from the diastereomers, was set as the base peak. In this case, the dehydrated ion was the base peak for *erythro*-Z-γ-Hy-D-Orn(Z) while the fragment ion at *m/z* 289 was the base peak for *threo*-Z-γ-Hy-L-Orn(Z). On the other hand, the $[M - H]^-$ ion was the base peak for both *erythro*-Z-γ-Hy-L-Orn(Z) and *threo*-Z-γ-Hy-D-Orn(Z). However, the abundance of the ion at *m/z* 289 in *threo*-Z-γ-Hy-D-Orn(Z) was much stronger than that of *erythro*-Z-γ-Hy-L-Orn(Z), and the abundance of the dehydrated ion for the former was stronger than that for the latter. Our data showed that it is possible to discriminate among the four γ-HyOrn diastereomers by the Z-group protection of the amino groups in these compounds in negative-ion FABMS.

The FAB (CAD) tandem mass spectra of the $[M - H]^-$ ion for the four Z-protected γ-HyOrn diastereomers are shown in Fig. 3(a)–(d). The dehydrated ion was not observed at all for *erythro*-Z-γ-Hy-L-Orn(Z), whereas it was seen with only weak abundance for the other three diastereomers. The stronger the abundance

Table 1. Relative intensities (%) of the peaks of the $[M - H]^-$ and fragment ions for four Z-protected γ-HyOrn diastereomers in negative-ion FABMS[a]

Compound	m/z 415	397	307	289	281	275
erythro-Z-γ-Hy-L-Orn(Z)	60 (100)	9 (15)	14 (23)	3.5 (6)	23 (38)	100
threo-Z-γ-Hy-L-Orn(Z)	1.5 (4)	38 (90)	9 (21)	42 (100)	2 (5)	100
erythro-Z-γ-Hy-D-Orn(Z)	5.5 (11)	51.5 (100)	34 (66)	29 (56)	5 (10)	100
threo-Z-γ-Hy-D-Orn(Z)	36 (100)	20 (56)	20 (56)	28 (78)	20 (56)	100

[a] The values in parentheses indicate the relative intensities of the ions when the ion with the strongest intensity was set as the base peak.

of the dehydrated ion becomes under FAB conditions, the stronger it becomes under CAD conditions. The formation of the dehydrated ion in the solution phase is analogous to that in the gas phase. These results indicate that the elimination of the hydroxyl group from each diastereomer can be attributed mainly to the structural differences of these diastereomers. It is therefore likely that the formation of the dehydrated ion may

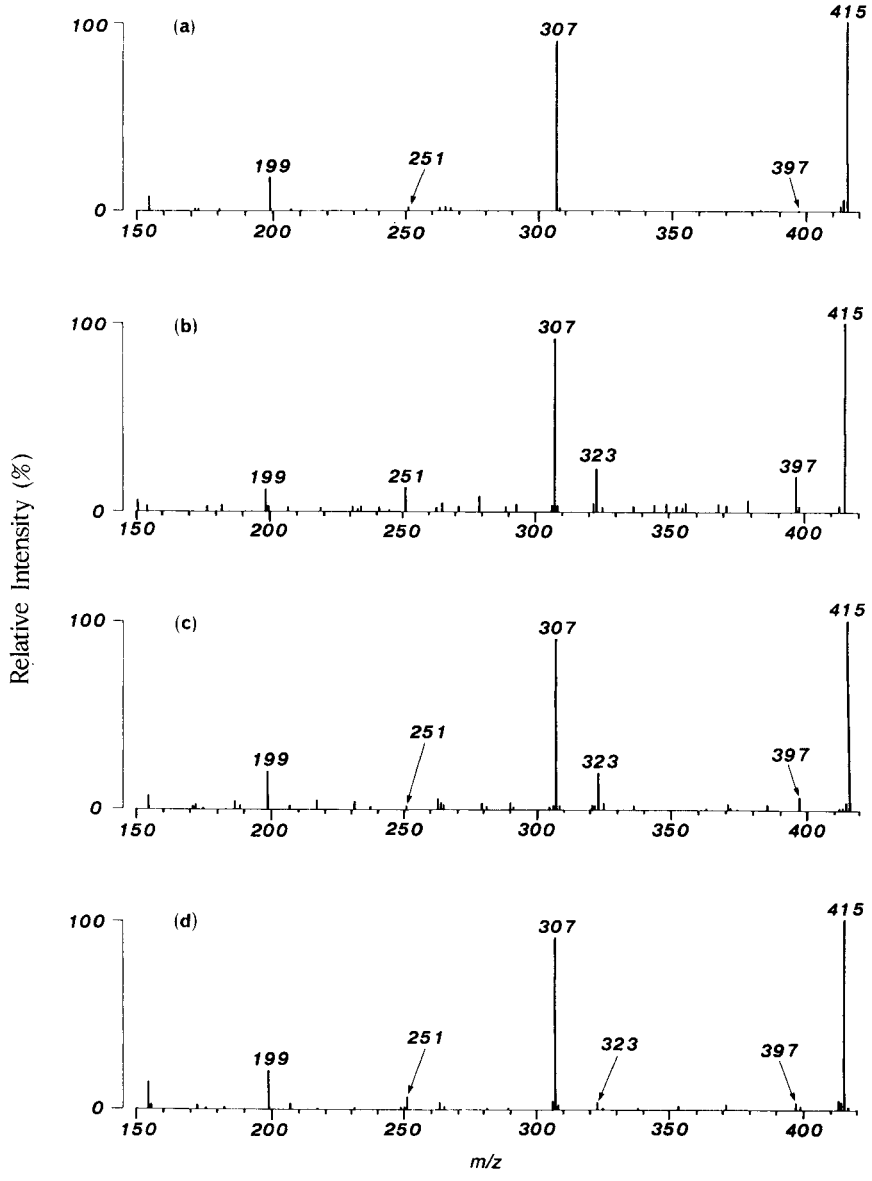

Figure 3. Negative-ion FAB (CAD) tandem mass spectra for four Z-protected γ-HyOrn diastereomers: (a) *erythro*-Z-γ-Hy-L-Orn(Z); (b) *threo*-Z-γ-Hy-L-Orn(Z); (c) *erythro*-Z-γ-Hy-D-Orn(Z); (d) *threo*-Z-γ-Hy-D-Orn(Z).

Table 2. Relative intensities (%) of the peaks of the main fragment ions for four Z-protected γ-HyOrn diastereomers in the negative-ion FAB (CAD) tandem mass spectra when the ion at *m/z* 307 was set as the base peak

Compound	*m/z* 397	323	251	307
erythro-Z-γ-Hy-L-Orn(Z)	ND[a]	ND[a]	2	100
threo-Z-γ-Hy-L-Orn(Z)	20	25	15	100
erythro-Z-γ-Hy-D-Orn(Z)	7	20	2	100
threo-Z-γ-Hy-D-Orn(Z)	5	5	7	100

[a] Not detected.

depend on the extent of the surroundings by the two benzene rings in the diastereomers. It is therefore thought that both *erythro*-Z-γ-Hy-L-Orn(Z) and *threo*-Z-γ-D-Orn(Z) were more surrounded by the benzene rings than *threo*-Z-γ-Hy-L-Orn(Z) and *erythro*-Z-γ-Hy-D-Orn(Z). Changes in the abundances of the several fragment ions, e.g. $[M - H - C_6H_5CH_2 - H]^-$ at *m/z* 323, $[M - H - C_6H_5CH_2O - H]^-$ at *m/z* 307, $[M - H - C_6H_5CH_2OCONHCH_2]^-$ at *m/z* 251 and $[307 - C_6H_5CH_2O - H]^-$ at *m/z* 199, were found in the CAD spectra for the four diastereomers. Table 2 shows the relative intensities of the peaks of the fragment ions when the ion at *m/z* 307 was set as the base peak. These results therefore allow us to differentiate among the four γ-HyOrn diastereomers by the CAD mass spectra.

CONCLUSIONS

Four γ-HyOrn diastereomers were discriminated by the Z-group protection of both the α- and δ-amino groups in γ-HyOrn in negative-ion FAB and CAD mass spectrometry. These results indicate that the orientation of the γ-hydroxyl group and the existence of two benzene rings on the Z groups are important factors responsible for the fragmentations of the four Z-protected γ-HyOrn diastereomers in negative-ion FABMS. Therefore, negative-ion FABMS of Z-protected γ-HyOrn diastereomers promises to be a useful method which can enable us to determine the configuration of each diastereomer of γ-HyOrn.

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