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Effects of compositions of dimethyl-β-cyclodextrins on enantiomer separations by cyclodextrin modified capillary zone electrophoresis

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Abstract

In enantiomeric separation by capillary zone electrophoresis using dimethyl-β-cyclodextrin (DM-β-CD) as a chiral selector, enantioselectivities were sometimes significantly different among DM-β-CDs from five different suppliers. The reason was due to the difference in the compositions among these commercial products, which was shown by the liquid chromatographic (LC) analysis. As for commercial DM-β-CD from one supplier, two major components were obtained by preparative LC. NMR spectroscopic and mass spectrometric analyses of these two components were performed to estimate the structure of each component. The results implied that the commercial products consist of heptakis(2,6-di-*O*-dimethyl)-β-CD and hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD. Sometimes different enantioselectivities were observed between these two components including the original DM-β-CD.

Keywords: Capillary zone electrophoresis; Cyclodextrins; CZE; CD-CZE; Enantiomer separation; Dimethyl-β-CD

1. Introduction

Capillary electrophoresis (CE), an instrumental version of electrophoresis, has been recognized as a useful separation technique in various analytical fields owing to its high resolving power and a number of papers on CE have appeared [1]. Also in the area of pharmaceutical sciences, CE has become one of the most effective separation techniques in terms of the micro scale and fast analysis [2], including chiral separations of drug enantiomers [3–5].

The separation of enantiomers is one of major objectives of CE as well as of chromatographic methods, especially in the pharmaceutical and biochemical areas. Among several methods used for enantiomeric separations by CE, the use of cyclodextrins (CDs) as chiral selectors in capillary zone electrophoresis (CZE) is one of the most

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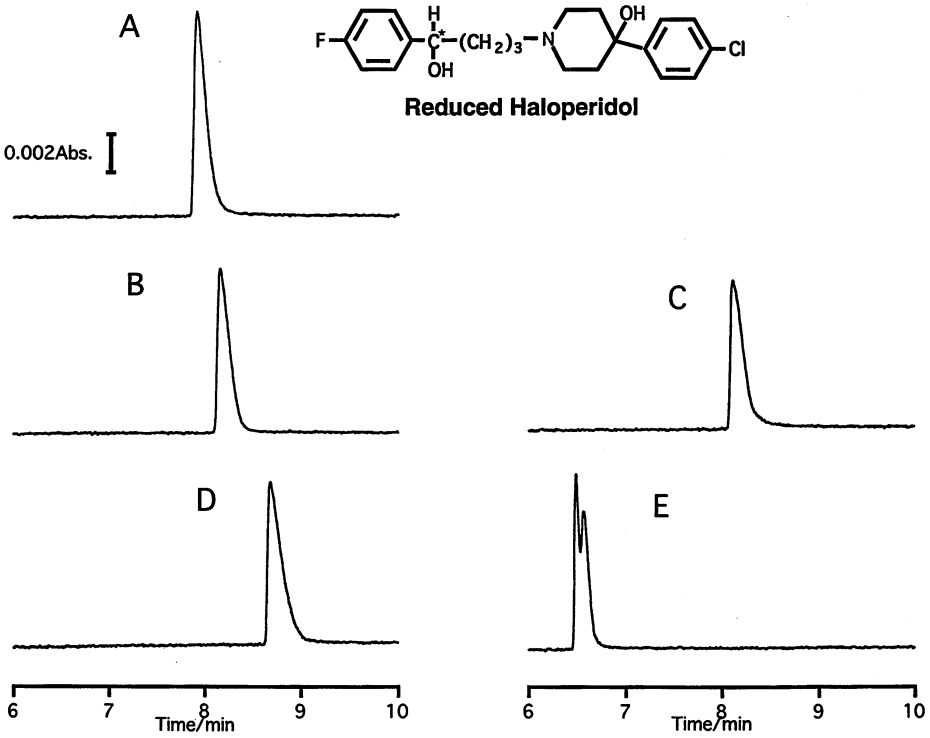

Fig. 1. Enantiomeric separations of RHP by CD-CZE using different DM-β-CDs obtained from five suppliers. Suppliers are abbreviated as A, B, C, D, and E (not in alphabetical order of the names of the suppliers). Separation solutions, 10 mM DM-β-CDs (pH 2.5); capillary, 50 μm i.d. × 300 mm; total applied voltage, 22 kV (295 V cm^{-1}); detection wavelength, 200 nm; temperature, 21°C.

popular technique, which is so-called CD modified CZE (CD-CZE). In CD-CZE, various CDs are used to achieve chiral recognition, including underivatized or natural CDs, e.g. α-, β-, and γ-CDs, neutral derivatized CDs, e.g. dimethyl- and trimethyl-β-CDs and hydroxypropyl-α-CD, and ionic derivatized CDs, e.g. carboxymethyl-β-CD and β-CD phosphate. Among these CDs, dimethyl-β-CD (DM-β-CD), which is neutral or non-charged CD and is commercially available as heptakis(2,6-di-*O*-methyl)-β-CD, has been frequently used and found to be effective for enantiomeric separation of various ionic drug components [4].

We have found that only one DM-β-CD obtained from one supplier could give a successful separation of reduced haloperidol (RHP) [6–8], whereas all other DM-β-CDs were not effective, as shown in Fig. 1. Similar results were more or less observed for other chiral drug components. These different enantioselectivities might be due to a difference in compositions among these commercially available DM-β-CDs, or the different substitution degree of methyl groups in each DM-β-CD. Koizumi et al. [9] reported that a commercial and two synthetic samples of DM-β-CDs consisted of two major components, i.e. heptakis(2,6-di-*O*-methyl)-β-CD and hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD. Weseloh et al. [10] synthesized several methylated β-CDs, including DM-β-CD, and investigated the enantioselectivities on some basic chiral drugs by CD-CZE using these CD derivatives as chiral selectors. They also found that the synthesized DM-β-CD contained 10% of hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD, 2.1% of hexakis(2,6-di-*O*-methyl)-mono(2-*O*-methyl)-β-CD, 0.8% of hexakis(2,6-di-*O*-methyl)-

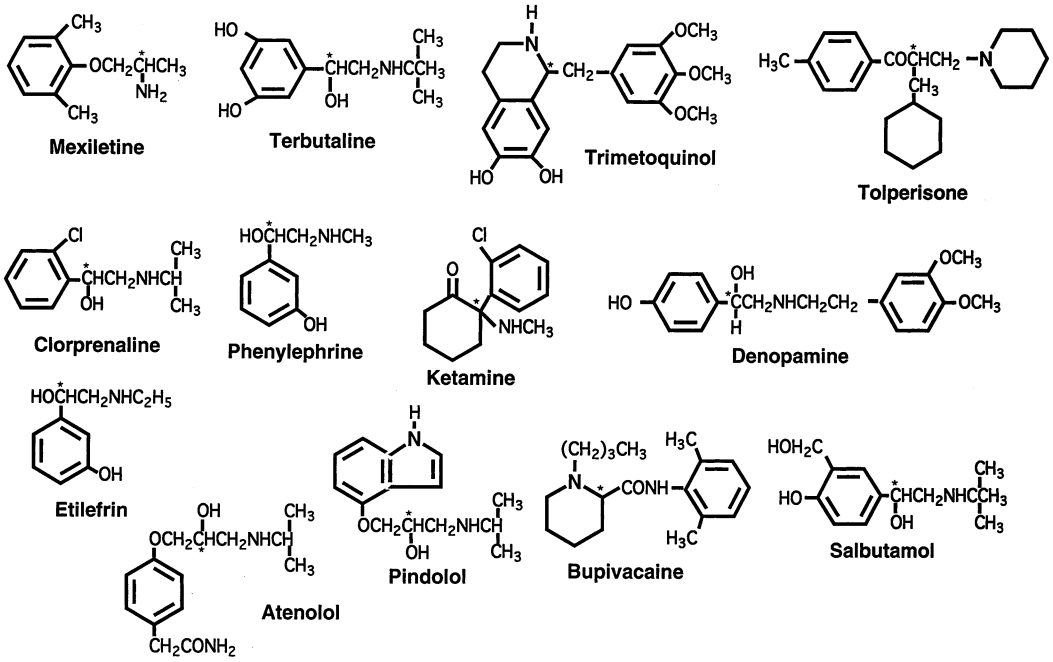

Fig. 2. Chemical structures of optically isomeric samples used in this work.

mono(2,3-di-*O*-methyl)-β-CD, and 0.5% of hexakis(2,6-di-*O*-methyl)-6-*O*-methyl-β-CD.

In this study, the difference in the compositions among five commercially available DM-β-CDs was investigated by liquid chromatographic (LC) analysis and isolation of the major components by preparative LC, followed by NMR spectroscopic and mass spectrometric (MS) analyses. One commercial DM-β-CD consisted of two major components or two methylated CDs of different substitution degree as mentioned above, whereas one DM-β-CD among the other four was found as a complex mixture. Effects of the difference in compositions on enantioselectivity for several chiral drugs are briefly presented.

2. Experimental

2.1. Chemicals and reagents

Dimethyl-β-cyclodextrins (DM-β-CDs) were purchased from five different suppliers, such as Beckman (Fullerton, CA), Sigma (St. Louis, MO), Nacalai Tesque (Kyoto, Japan), Funakoshi (Tokyo, Japan), and Aldrich (Milwaukee, WI), which are supplied as heptakis(2,6-di-*O*-methyl)-β-cyclodextrins. As racemic samples, we mainly used several drug components, as shown in Fig. 2. Sample solutions were prepared by dissolving each compound in water or water–acetonitrile at the concentration of ca. 0.1 mg ml^{-1}. All other reagents used were of analytical grade. Water was purified with a Milli-Q Labo system (Nihon Millipore, Yonezawa, Japan).

Separation solutions were prepared by dissolving DM-β-CD at 10 mM in 40 mM phosphate buffer (pH 2.5) and filtered through a 0.2 μm membrane filter prior to use.

2.2. Instrumentation

Capillary electrophoresis was performed by a Beckman P/ACE system 2200 equipped with a UV detector. The operation of the CE instrument and data processing was carried out by the P/ACE Station software running on Windows 95 or the System Gold software on MS-DOS. The sepa-

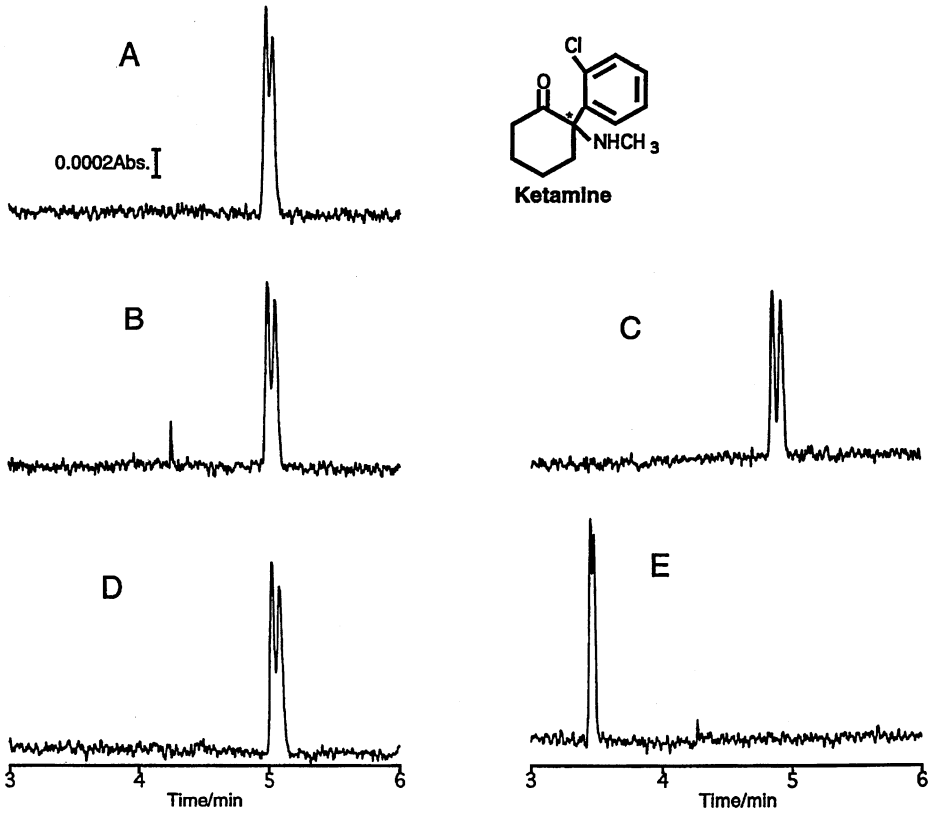

Fig. 3. Enantiomeric separations of ketamine by CD-CZE with five different DM-β-CDs. Conditions as in Fig. 1.

ration capillary used was an untreated fused silica tubing of 50 μm i.d. × 370 mm (300 mm effective) obtained from Polymicro Technologies (Phoenix, AZ).

Proton and carbon-13 NMR spectra were recorded on a JNM-GX400 FT-NMR spectrometer (JEOL, Akishima, Tokyo, Japan). Matrix-assisted laser desorption ionization (MALDI)/time-of-flight (TOF) mass spectra were recorded on a Reflex II mass spectrometer (Bruker Japan, Tsukuba, Ibaraki, Japan).

3. Results and discussion

3.1. Commercial DM-β-CDs

Fig. 3 shows enantiomer separations of ketamine by CD-CZE using five different DM-β-CDs, under the same separation conditions as in Fig. 1. Different from the case of RHP (Fig. 1), the ketamine enantiomers were successfully separated by any of the five DM-β-CDs.

Fig. 4 summarizes the enantioselectivities of the five DM-β-CDs for each enantiomer in CD-CZE. Here, the enantioselectivity is represented by an α value, which is defined as the ratio of the effective mobilities of the enantiomers 1, μ(eff, 1), and 2, μ(eff, 2):

$$\alpha = \mu(\text{eff, 2})/\mu(\text{eff, 1}) \quad (1)$$

The effective mobility of enantiomer 1 is given by

$$\mu(\text{eff, 1}) = \mu(1) - \mu(\text{eof}) \quad (2)$$

where $\mu(1)$ and μ(eof) are the apparent mobility of the enantiomer 1 and electroosmotic mobility, respectively. Then, α is calculated by

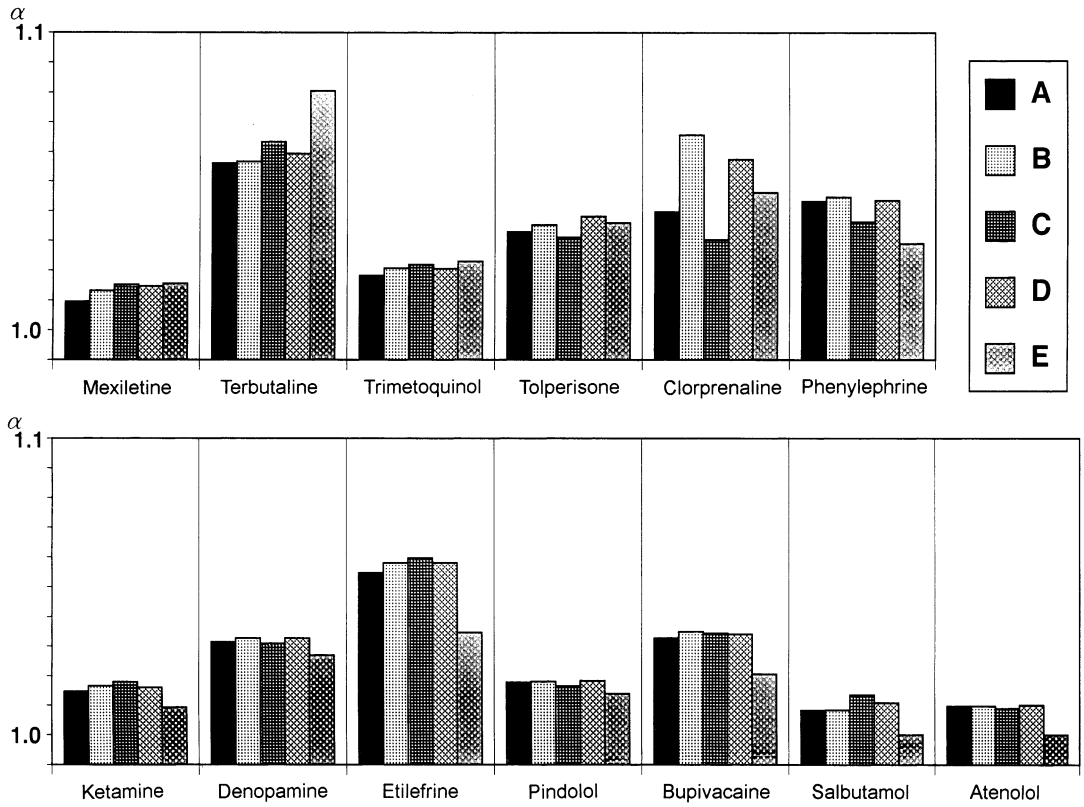

Fig. 4. Differences in enantioselectivities, α, among five DM-β-CDs for each solute. Separation conditions as in Fig. 1. In each graph for each sample, five bars from left to right correspond to suppliers A, B, C, D, and E, respectively.

$$\alpha = [t(1)\ \{t(\text{eof}) - t(2)\}]/[t(2)\ \{t(\text{eof}) - t(1)\}] \quad (3)$$

where $t(1)$, $t(2)$, and $t(\text{eof})$ are the migration times of the isomers 1, 2, and the marker of the electroosmotic flow (EOF), respectively. Thiourea was used as the EOF marker. In this experiment, the optical isomers or the samples are basic compounds and hence, under the acidic conditions used their mobilities are larger than the electroosmotic mobility. We can recognize slightly different enantioselectivities among these five DM-β-CDs. As mentioned above, although the RHP racemate was optically resolved only by DM-β-CD(E), this CD was not always superior to other four DM-β-CDs in terms of the enantioselectivities. For example, an atenolol racemate was not resolved with DM-β-CD(E) or the α value was unity, while other four DM-β-CDs gave better enantioseparations ($\alpha = 1.1$), as shown in Fig. 4.

These differences in enantioselectivities must be due to a different characteristic of each DM-β-CD or a different substitution degree of methyl group in each DM-β-CD. To confirm the differences among these five DM-β-CD compositions, we first tried to analyze them by high performance liquid chromatography (HPLC). The separation was performed by using an amino bonded phase $5NH_2$-MS column of 4.6 mm i.d. $\times$ 150 mm (Nacalai Tesque) and differential refractive index (RI) detection with acetonitrile–water (99: 1) as a mobile phase. Results of the HPLC analyses of the five DM-β-CDs are shown in Fig. 5. From the chromatographic point of view, compositions of four DM-β-CDs from the suppliers, A (DM-β-CD(A)), B (DM-β-CD(B)), C (DM-β-CD(C)), and D (DM-β-CD(D)) are similar to each other, whereas DM-β-CD(E) is significantly different from the other four. Most α values in Fig. 4 with

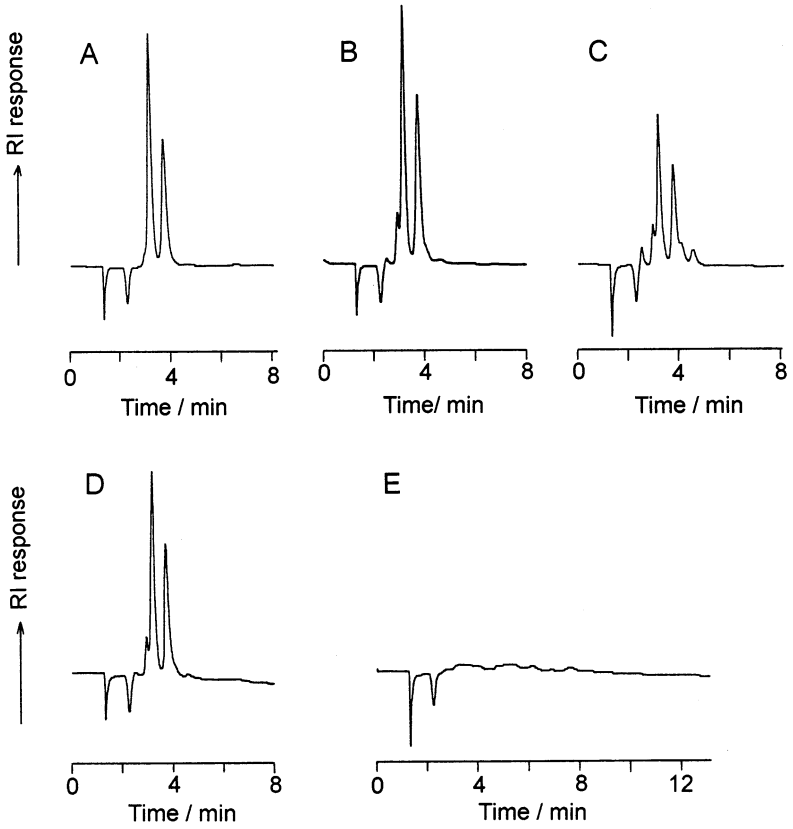

Fig. 5. HPLC analyses of the five DM-β-CDs. Conditions, see text.

DM-β-CD(A), DM-β-CD(B), DM-β-CD(C), and DM-β-CD(D) are similar to each other, whereas the α values with DM-β-CD(E) are sometimes much different from the other four DM-β-CDs. These are in good agreement with the liquid chromatographic results. However, the enantioselectivities of the former four DM-β-CDs are not always similar to each other (e.g. clorprenaline), and the enantioselectivity of DM-β-CD(E) is not always different from the other four (e.g. tolperisone), as shown in Fig. 4.

3.2. Analyses of components of DM-β-CD(D) and DM-β-CD(E)

Then, the difference in compositions between DM-β-CD(D) and DM-β-CD(E) was investigated. As for DM-β-CD(D), preparative HPLC was carried out to obtain two fractions as shown in Fig. 6(a) by using a Cosmosil 5SL silica gel preparative column (Nacalai Tesque) of 10 mm i.d. $\times$ 500 mm with ethanol–ethyl acetate (20:80) as a mobile phase, followed by an HPLC analysis of the two fractions with a Cosmosil 5SL (4.6 mm i.d. $\times$ 250 mm) and the same mobile phase as the preparative run. The results of the HPLC analysis are shown in Fig. 6(b). We can see the successful fractionations of two major components of DM-β-CD(D).

On the other hand, DM-β-CD(E) was fractionated into three, as shown in Fig. 7(a), although each component was not clearly separated. Similarly, the three fractions were analyzed by silica gel HPLC and the results are shown in Fig. 7(b). Obviously, each fraction seems to be a mixture or unpurified.

By using the above five fractions, two from DM-β-CD(D) and three from DM-β-CD(E), the

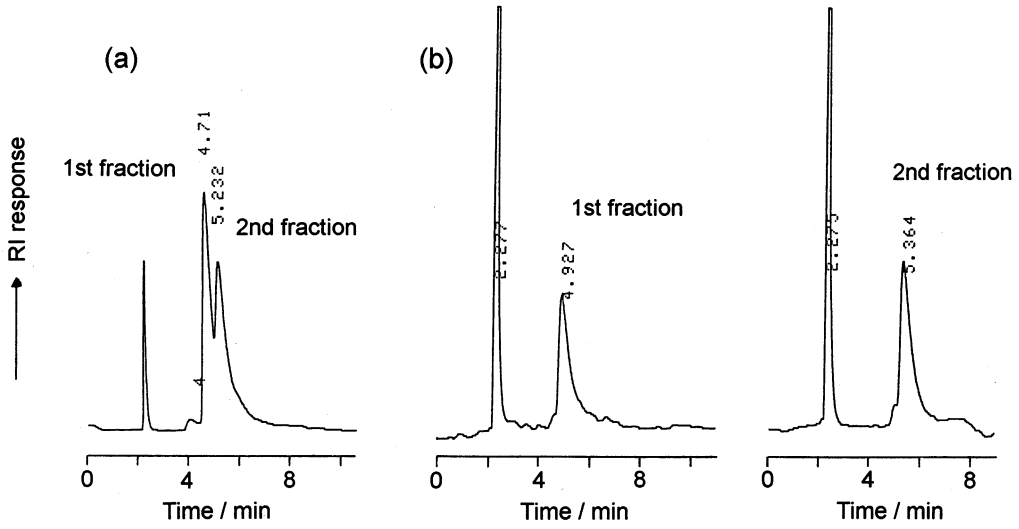

Fig. 6. Preparative HPLC of DM-β-CD(D) into two fractions (a) and HPLC analyses of the two fractions (b). Conditions, see text.

same CD-CZE experiments of separating optical isomers were carried out as in Fig. 1 and Fig. 3. The results are summarized in Fig. 8, including the results obtained with the original DM-β-CD(D) and DM-β-CD(E). Similar to the case in Fig. 4, it is not easy to systematically explain these different enantioselectivities.

To identify the compositions of the two fractions obtained from DM-β-CD(D), proton and carbon-13 NMR and MALDI-TOF mass spectrometry (MS) were employed.

As for the first eluted peak or first fraction of DM-β-CD(D), we could assign the component as heptakis(2,6-di-*O*-methyl)-β-CD. The proton NMR spectrum at 400 MHz in ^2H_2O, as shown in Fig. 9 where sodium 3-(trimethylsilyl)propionate (TSP) was used as an internal standard, is equivalent to that reported by Yamamoto et al. [11]. The carbon-13 NMR spectrum at 100 MHz in ^2H_2O (TSP as an internal standard), as shown in Fig. 10, is also equivalent to those in the references [11,12]. Both proton- and carbon-13 NMR chemical shifts for the first fraction of DM-β-CD(D) are summarized in Table 1, together with those which appear in the literature [11]. All the chemical shifts obtained in this work are well consistent with those in the reference. Also the spectrum of carbon-13 NMR well corresponds to that reported [12]. MALDI-TOF-MS analysis showed that the molecular mass of the first fraction is 1330 since the value of m/z of the major peak (1338) corresponds to that of the protonated complex of the first fraction with Li, $[M + Li + H]^+$, as shown in Fig. 11. The molecular mass of heptakis(2,6-di-*O*-methyl)-β-CD is 1330 and hence, the result strongly supports this assignment.

Similar evaluation was attempted for confirming the composition of the second fraction of DM-β-CD(D). Compared with the first fraction, however, both the proton and carbon-13 NMR spectra were not clear, probably due to low purity of the fraction obtained by the preparative HPLC. According to Koizumi et al. [9,12], we can assume that the second fraction is hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD. Although the spectra of proton and carbon-13 NMR of the second fraction could not be fully assigned as such the component (data not shown), the result from MALDI-TOF-MS strongly suggests the possibility of the structure, since the value of m/z of major peak (1352) corresponds to $[M + Li + H]^+$, as shown in Fig. 12, where the molecular mass of hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD is 1344, similar to the first fraction mentioned above.

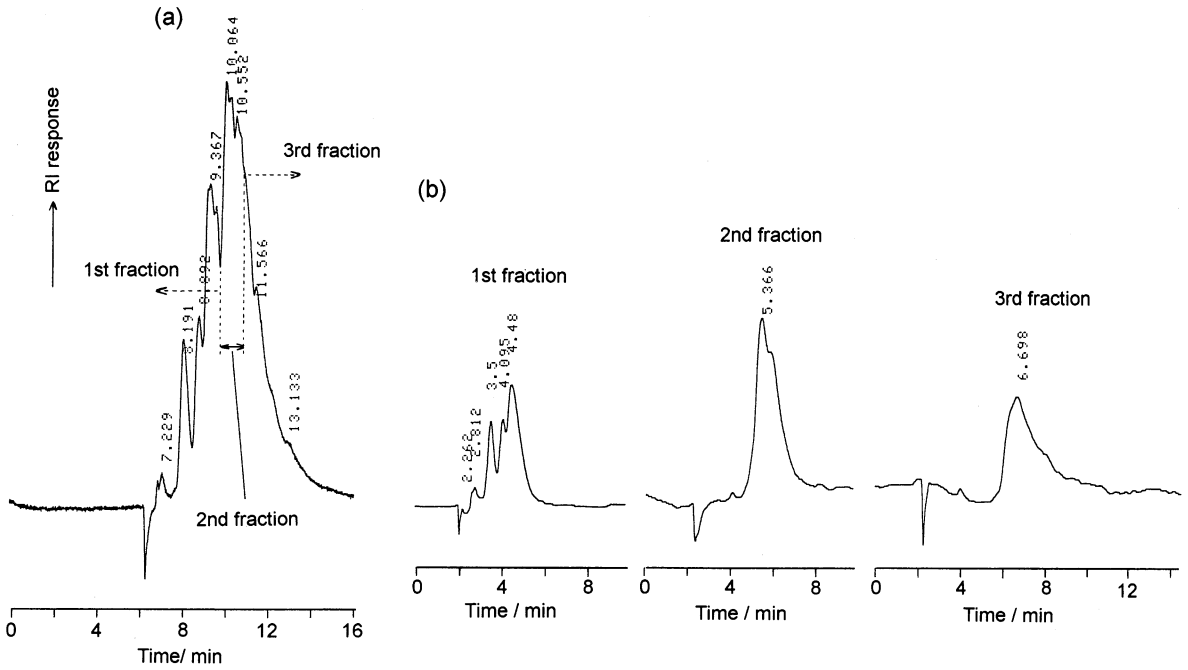

Fig. 7. Preparative HPLC of DM-β-CD(E) into three fractions (a) and HPLC analyses of the two fractions (b). Conditions, see text.

These two major components of DM-β-CD(D) and the original DM-β-CD(D) itself generally show slightly different enantioselectivities to each other for the chiral solutes used in this study, as shown in Fig. 8. Except for mexiletine, all the solutes could be optically resolved by using these three CDs. However, sometimes hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD is superior to the other two CDs in terms of the α values. Especially for mexiletine, terbutaline, trimetoquinol, and tolperisone, this CD shows higher α values than heptakis(2,6-di-*O*-methyl)-β-CD and slightly better than the original DM-β-CD(D). This implies that the use of the pure hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD and/or the pure heptakis(2,6-di-*O*-methyl)-β-CD in CD-CZE can be expected to give higher enantioselectivities and higher reproducibilities for many optical isomers rather than using a commercial DM-β-CD itself.

4. Conclusion

Although the results in this paper are preliminary, we can conclude that commercially available DM-β-CDs consist of two or more components of methylated β-CDs. Except for DM-β-CD(E), all four DM-β-CDs used in this work probably contain two major components from the view point of HPLC analyses. The major components in DM-β-CD(D) are estimated to be heptakis(2,6 - di - *O* - methyl)-β-CD and hexakis(2,6-di-*O*-methyl)-mono(2,3,6-tri-*O*-methyl)-β-CD. The en-antioselectivities in CD-CZE achieved by using the commercial DM-β-CD(D) and its two components mentioned above are different. By using the pure CD derivatives we can expect to obtain better enantioseparations for some optical isomers and better reproducibilities.

To evaluate the effect of the compositions on enantioselectivity, binding constants should be calculated [13,14]. Calculation of binding constants with the first and second fractions of DM-β-CD(D) has been in progress [15], and the results will appear elsewhere.

Acknowledgements

The authors are grateful to Professor Hsin-

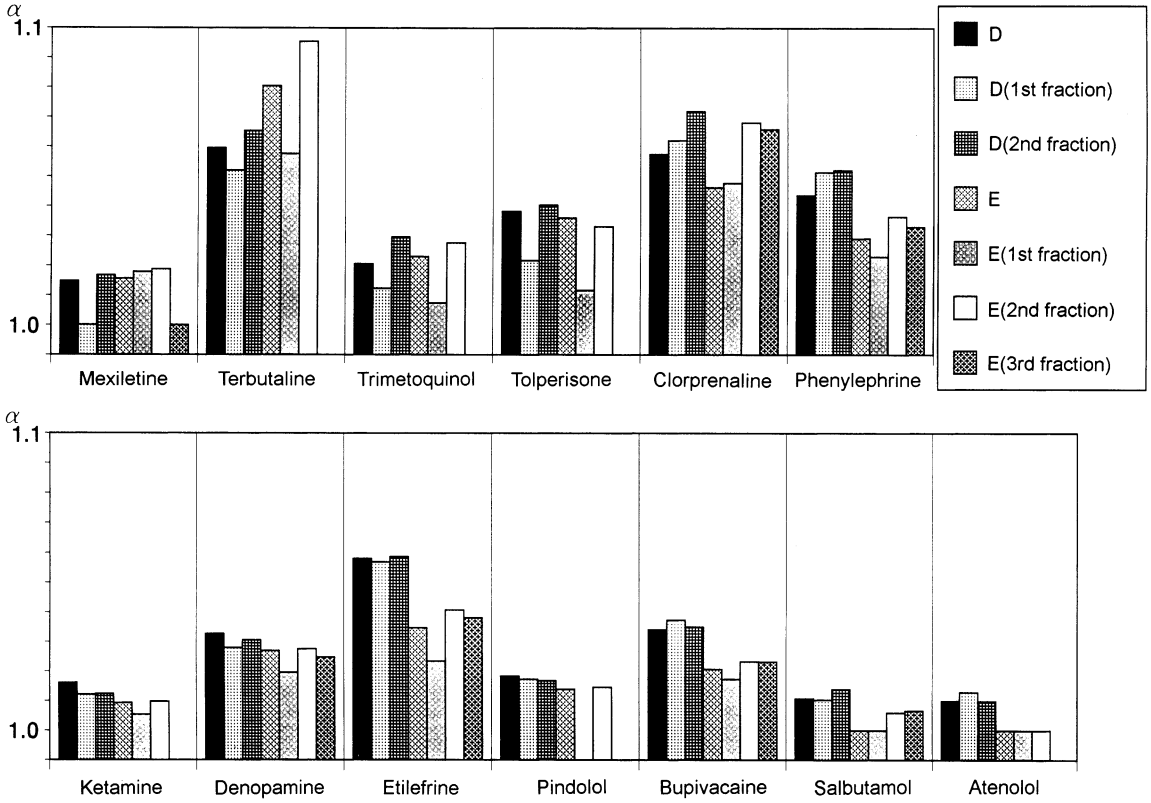

Fig. 8. Differences in enantioselectivities, α, among DM-β-CD, two components of DM-β-CD(D), DM-β-CD(E), and three components of DM-β-CD(E). Conditions as in Fig. 4. In each graph for each sample, seven bars from left to right correspond to DM-β-CD(D), the first fraction of DM-β-CD(D), the second fraction of DM-β-CD(D), DM-β-CD(E), the first fraction of DM-β-CD(E), the second fraction of DM-β-CD(E), and the third fraction of DM-β-CD(E), respectively.

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Table 1
Proton and carbon-13 NMR chemical shifts[a] for the fraction 1 of DM-β-CD(D)

Proton in ^2H_2O								
	H-1	H-2	H-3	H-4	H-5	H-6	2-OCH_3	6-OCH_3
Found	5.24	3.37	3.97	3.61	3.89	3.73	3.39	3.56
Ref[b]	5.18	3.37	3.94	3.55	3.85	3.72	3.36	3.55
Carbon-13 in ^2H_2O								
	C-1	C-2	C-3	C-4	C-5	C-6	2-OCH_3	6-OCH_3
Found	101.1	82.8	73.8	83.5	71.3	72.0	59.6	60.8
Ref[b]	99.9	81.7	72.7	82.3	70.3	71.0	58.6	59.7

[a] Internal standard, sodium 3-(trimethylsilyl)propionate.
[b] From [11]. Internal standard, tetramethylsilane.

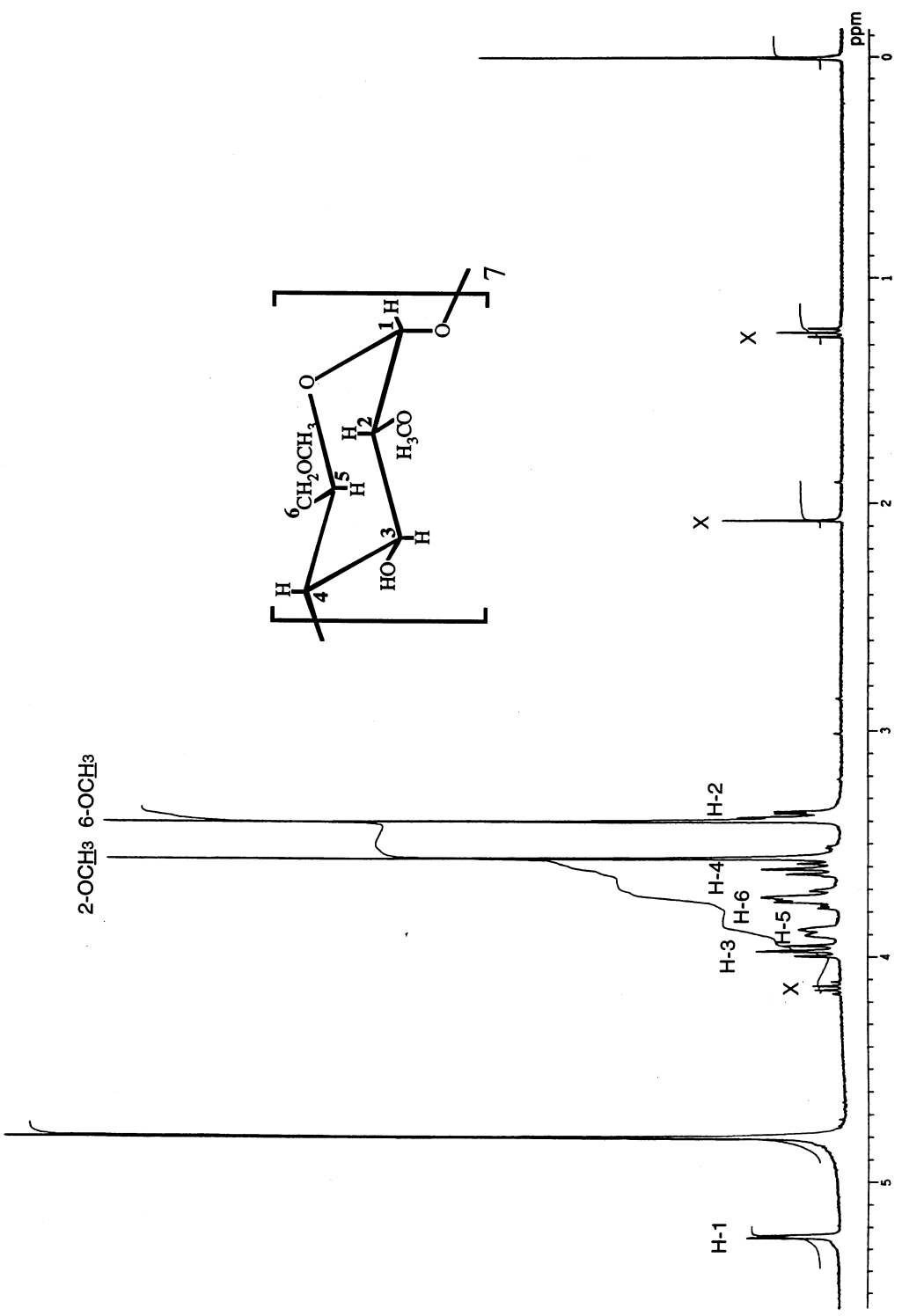

Fig. 9. The proton NMR spectrum at 400 MHz in ^2H_2O and peak assignment of the first fraction of DM-β-CD(D). TSP was used as an internal standard for the chemical shift measurement. Peaks marked × are of ethyl acetate from preparative HPLC.

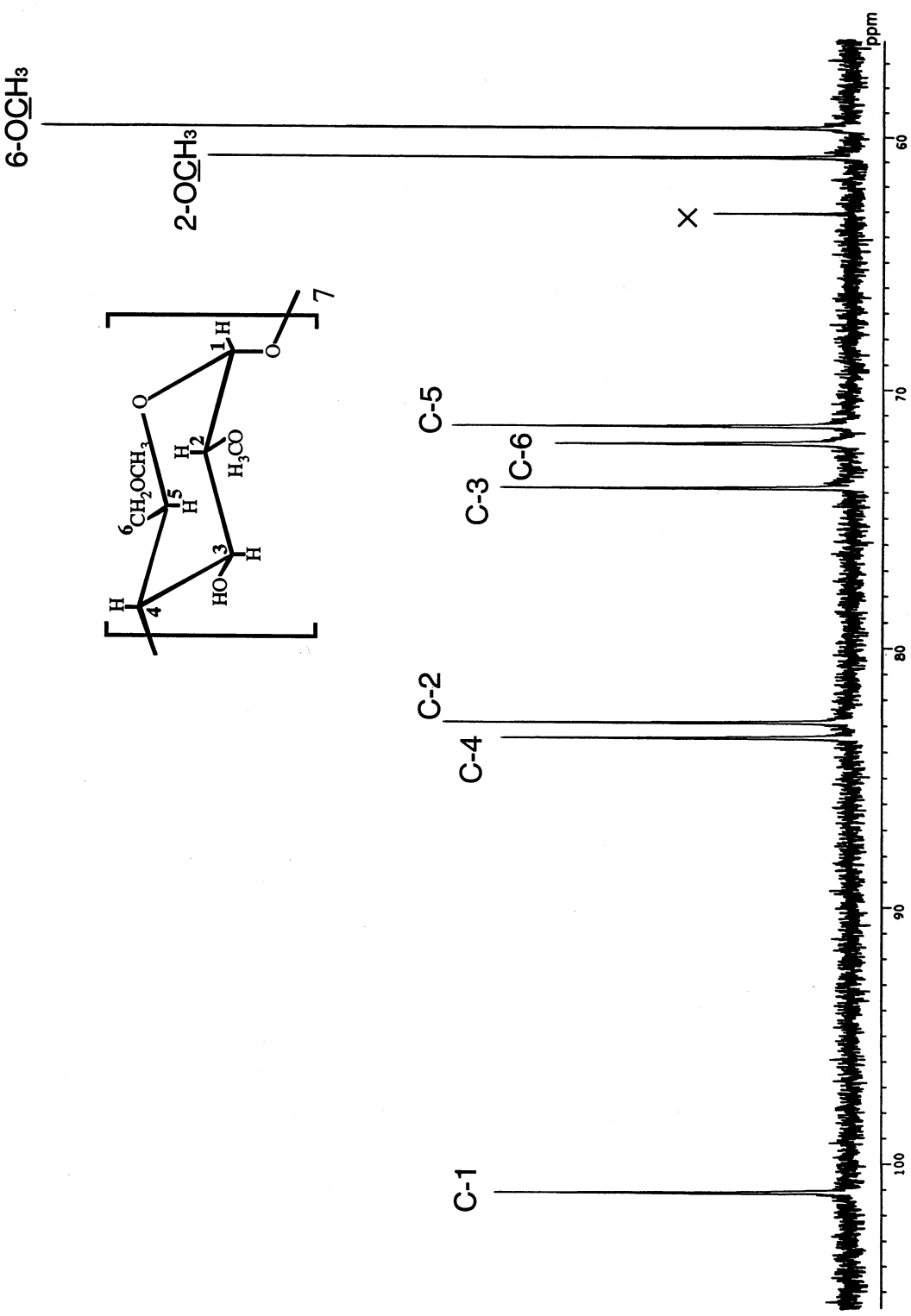

Fig. 10. The carbon-13 NMR spectrum at 100 MHz in ^2H_2O and peak assignment of the first fraction of DM-β-CD(D). TSP as an internal standard.

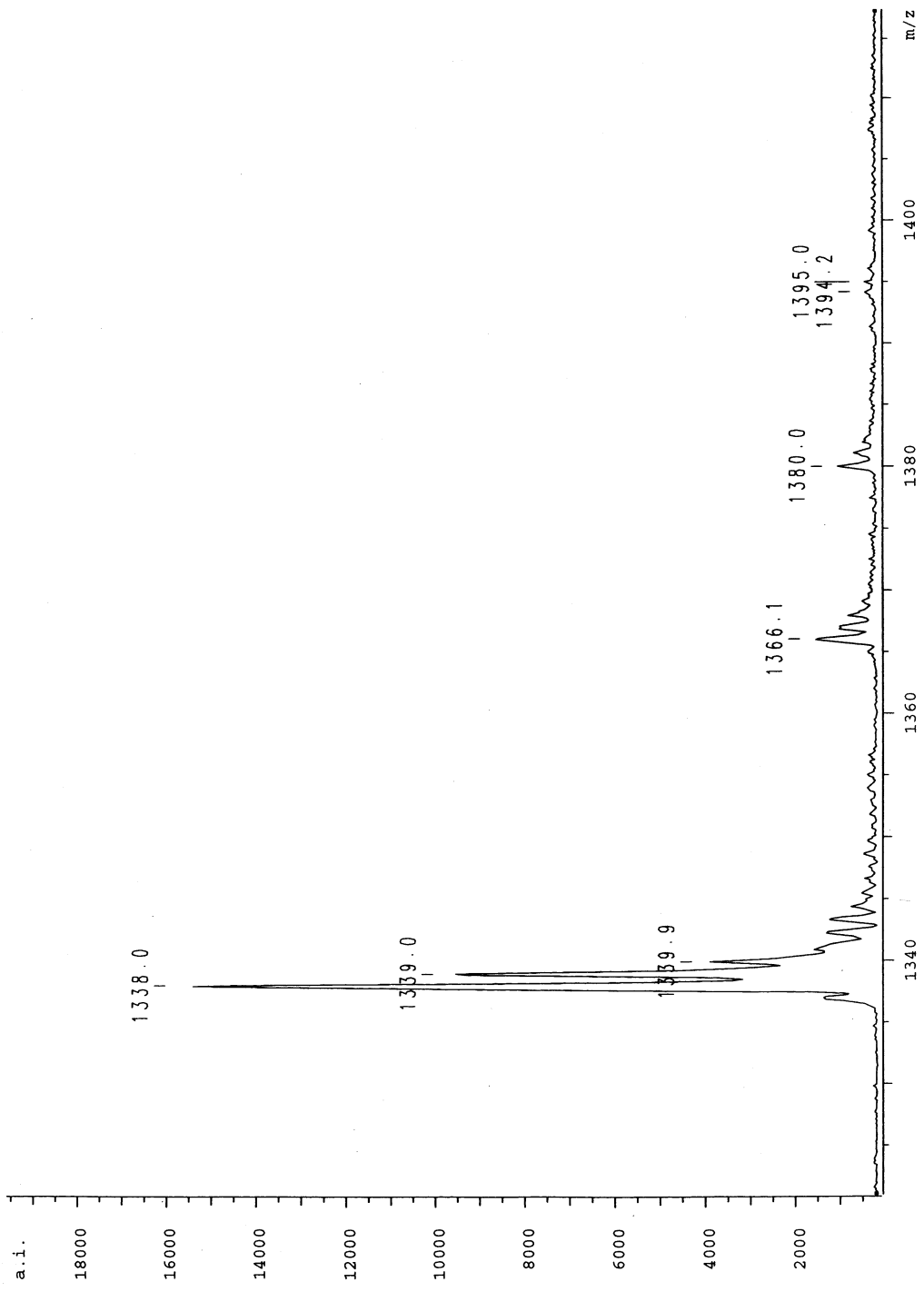

Fig. 11. The MALDI-TOF-MS spectrum of the first fraction of the DM-β-CD(D). Matrix, α-cyano-4-hydroxcinnamic acid/lithium trifluoromethanesulfonate.

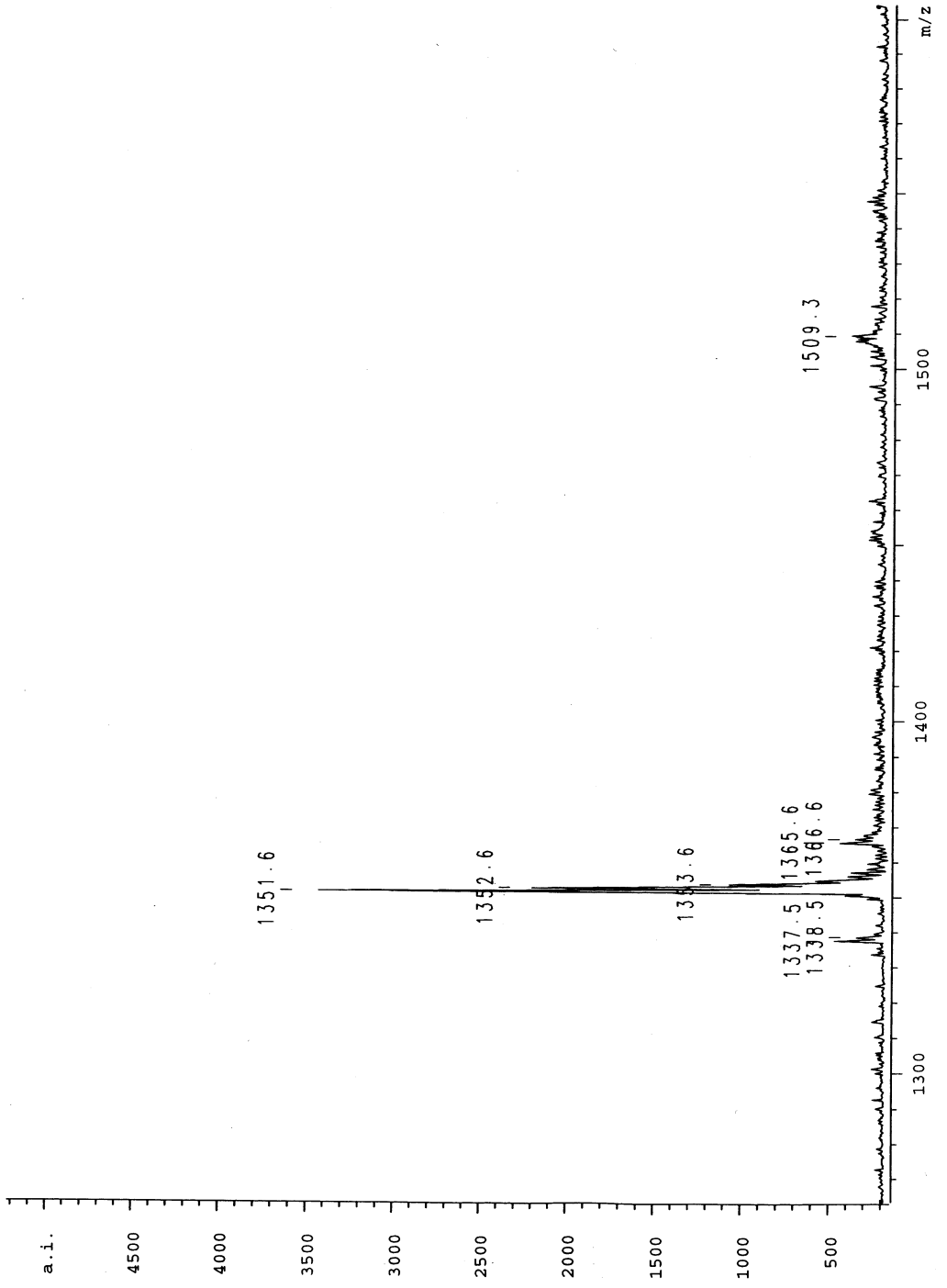

Fig. 12. The MALDI-TOF-MS spectrum of the second fraction of DM-β-CD(D).