Carbohydrate Research, 222 (1991) 23–35
Elsevier Science Publishers B.V., Amsterdam

Analysis of cyclomalto-oligosaccharides (cyclodextrins) and derivatives thereof by ion-spray mass spectrometry

Jörg W. Metzger, Martin Jung, Dieter Schmalzing, Ernst Bayer, and Volker Schurig*
Institut für Organische Chemie der Universität Tübingen, Auf der Morgenstelle 18, D-7400 Tübingen (Germany)

(Received December 18th, 1990; accepted for publication March 28th, 1991)

ABSTRACT

The use of ion-spray (i.s.) mass spectrometry for the rapid determination of molecular weight and purity is demonstrated for derivatives of cyclomalto-hexaose (αCD), -heptaose (βCD), and -octaose (γCD), and for βCD. The i.s.-mass spectra contain peaks for singly and doubly charged protonated and cationised quasi-molecular ions. Methylation of CDs yielded homogeneous compounds, whereas the synthesis of CDs containing methyl groups and an additional substituent, *e.g.*, allyl, pentenyl, octenyl, trifluoroacetyl, or heptafluorobutanoyl, afforded mixtures of products, the compositions of which could be determined directly from the i.s.-mass spectra. The addition of lithium chloride usually facilitated the interpretation of the spectra by producing abundant $(M + Li)^+$ ions and suppressing fragmentation. Zinc acetate favoured the formation of doubly charged molecular ions. Additional information on the structure of derivatised CDs can be obtained by i.s.-tandem mass spectrometry.

INTRODUCTION

Ion spray, pneumatically assisted electrospray[1], is a soft method of ionisation for non-volatile and thermolabile molecules. The method is based on the ion-evaporation process: when a solution of sample is sprayed through a capillary that is maintained at a few kilovolts relative to the entrance of the mass spectrometer, charged droplets are produced, from which the preformed solute ions are emitted directly into the gas phase at ambient temperature and atmospheric pressure. A characteristic of atmospheric-pressure ionisation (a.p.i.) is the occurrence of multiply charged quasi-molecular ions $(M + nH)^{n+}$, which allows the determination of masses that exceed the mass range of conventional quadrupole spectrometers. In general, the maximum number of charges of an ion obtained by ion evaporation is determined by the number of basic centers (mainly nitrogen and oxygen) in the molecule[2].

Alkylated cyclomalto-oligosaccharides (cyclodextrins, CDs), *e.g.*, heptakis-(2,3,6-tri-*O*-methyl)-βCD or heptakis(2,3,6-tri-*O*-pentyl)-βCD, have been employed[3–6] as chiral stationary phases for the resolution of racemates by high-resolution capillary g.l.c. Regioselectively derivatised CDs are also accessible[7], *e.g.*, 3-acyl-2,6-dialkyl-CDs,

---

* To whom correspondence should be addressed.

0008-6215/91/$ 03.50 © 1991 – Elsevier Science Publishers B.V. All rights reserved.

which are also useful chiral stationary phases[6–9]. There is an analytical need to determine the degree of alkylation and purity of derivatised CDs. Involatility, polarity, and high molecular weight render CDs and their derivatives unsuitable for conventional mass spectrometry and there have been a few reports in the literature. The purity of *n*-pentylated CDs used in g.l.c. has been assessed only indirectly by g.l.c.–m.s. of the glucose monomers obtained by reductive cleavage with triethylsilane in the presence of a Lewis acid[10]. However, various mass spectrometry techniques can be applied to intact CDs and their derivatives, such as fast-atom bombardment[11–13], secondary ion[14], laser-assisted field desorption[15], laser desorption[16], and electron ionisation–flash desorption[17]. The chiral stationary phase for g.l.c., permethylated *O*-(*S*)-2-hydroxypropyl-*β*CD, has been subjected to ^{252}Cf-plasma-desorption mass spectrometry, and analysis of the parent ion peaks led to an accurate evaluation of the components[5]. Recently, *α*-, *β*-, and *γ*-CD have been analysed by atmospheric-pressure spray-ionisation, a combination of thermospray and atmospheric-pressure ionisation[18]. Many of the above techniques use home-built mass spectrometers and ion sources, which are not available for routine analysis, and require a complicated and often difficult procedure for the preparation of samples.

We now report on the use of ion-spray(i.s.)-mass spectrometry and i.s.-tandem mass spectrometry for the determination of the molecular weight, purity, and structure of CDs and their derivatives. The advantages of this method are easy preparation of samples, short times of analysis, and the possibility of analysing compounds of high molecular weight on the basis of the interpretation of multiply charged ions.

EXPERIMENTAL

I.s.-mass spectra and i.s.-tandem mass spectra were recorded with a Sciex API III triple-quadrupole mass spectrometer with mass range of 2400 Da equipped with an ion-spray ion source (Sciex, Toronto, Canada). The mass spectrometer was operated under conditions of unit-mass resolution, and profile spectra were obtained by acquiring data points every 0.1 Da. The ion-spray voltage was 5 kV. *β*CD was dissolved in water–methanol (1:1), and the more lipophilic derivatives were dissolved in chloroform–methanol–aqueous 10% formic acid (2:3:1; for some solutions, the formic acid was replaced by aqueous 1% ammonium acetate). Inorganic salts were added as 10mM aqueous solutions. The final concentrations of substrate and salt were ~100μM. The solution was introduced into the ion-spray source at a constant flow rate between 2–10 μL/min with a μL syringe, using a medical infusion pump (Harvard Apparatus, U.S.A.). The total sample consumed was in the pmol range. Argon at ~1.3 × 10^{14} atoms.cm^{-2} was the collision gas for tandem mass spectrometry. The collision energies used were in the range 30–60 eV.

The *α*-, *β*- and *γ*-CDs were obtained from Fluka. The CD derivatives **3**, **7**, **8**, and **10**–**12** were prepared as described: heptakis(2,3,6-tri-*O*-methyl)-*β*CD[3,19,20] (**3**); heptakis-(2,6-di-*O*-methyl)-*β*CD (**7**) and heptakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*β*CD (**8**)[19,20]; and heptakis(*O*-allyl-*O*-methyl)-*β*CD (**10**), heptakis(*O*-methyl-*O*-4-pentenyl)-

βCD (**11**), and heptakis(*O*-methyl-*O*-7-octenyl)-βCD (**12**)[21]. Heptakis(2,3,6-tri-*O*-methyl)-α- (**2**) and -γ-CD (**4**) and the deuterated analogue (**5**) were prepared as for **3**. Heptakis(2,3,6-tri-*O*-pentyl)-βCD (**6**) was prepared as for **3** by alkylation with pentyl bromide. Heptakis(3-*O*-heptafluorobutanoyl-2,6-di-*O*-methyl)-βCD (**9**) was prepared as for **8**[22].

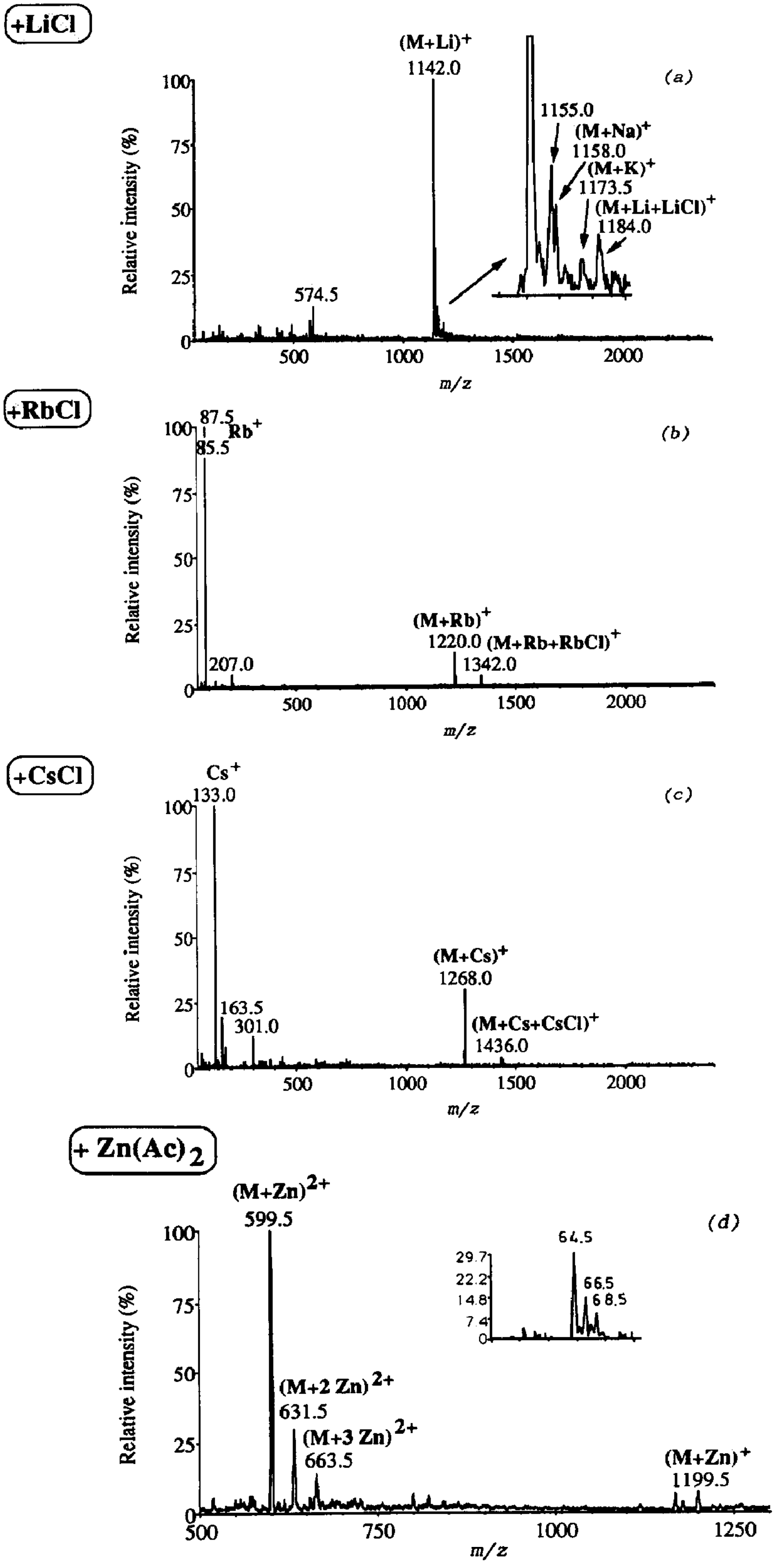
RESULTS AND DISCUSSION

The following CD derivatives were investigated: βCD (**1**, mol. wt. 1135.0), hexakis(2,3,6-tri-*O*-methyl)-αCD (**2**, 1224.9), heptakis(2,3,6-tri-*O*-methyl)-βCD (**3**, 1429.0), octakis(2,3,6-tri-*O*-methyl)-γCD (**4**, 1633.0), heptakis(2,3,6-tri-*O*-trideuteriomethyl)-βCD (**5**, 1492.0), heptakis(2,3,6-tri-*O*-pentyl)-βCD (**6**, 2605.0, not pure), heptakis(2,6-di-*O*-methyl)-βCD (**7**, 1331.0, not pure), heptakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-βCD (**8**, 2003.1, not pure), heptakis(3-*O*-heptafluorobutanoyl-2,6-di-*O*-methyl)-β-CD (**9**, 2703.5, not pure), *O*-allyl-*O*-methyl-βCD (**10**), *O*-methyl-*O*-4-pentenyl-βCD (**11**), and *O*-methyl-*O*-7-octenyl)-βCD (**12**). Based on the methods of synthesis, **1**–**5** should be pure compounds, whereas **6**–**12** were mixtures.

The intensities of the peaks observed in i.s.-mass spectra depended on the electronic parameters of the ion-spray interface. An increase in the voltage of the orifice, for example, enhanced the intensity of the singly charged ion and lowered that of the doubly charged ion. The mass spectra allow only a qualitative determination of the composition of a mixture. A quantitative determination is possible if calibration curves (obtained with mixtures of known composition) are used.

βCD (**1**). — The i.s.-mass spectrum of βCD (**1**) contained the ions $(M + H)^+$, $(M + Na)^+$, and $(M + K)^+$ with low intensities and fragments which could not be assigned unequivocally (data not shown). The natural levels of Na^+ and K^+ impurities in the samples are sufficient to produce these ions. However, the addition of inorganic salts markedly increased the intensity of the quasi-molecular peaks and suppressed the formation of fragments. The formation of adducts has also been observed[23] in plasma-desorption mass spectrometry after the addition of sodium and caesium salts to αCD. After the addition of lithium chloride, $(M + Li)^+$ was the base peak in the i.s.-mass spectrum and the doubly charged ion $(M + 2Li)^{2+}$ was observed (Fig. 1*a*). Similar results were obtained after addition of rubidium chloride and caesium chloride (Figs. 1*b* and *c*). The most abundant peaks in these spectra corresponded to Cs^+ and Rb^+, and even the two isotopes of Rb^+ could be distinguished. Furthermore, the salts MeX (LiCl, KI, RbCl, CsCl, and $AgNO_3$) form adduct ions $(M + Me + n\text{MeX})^+$, *e.g.*, $(M + Cs + CsCl)^+$, in which the charge of the additional cations is compensated by the corresponding counter ion, leaving a net charge of one or two (Figs. 1*a*–*c*).

The addition of zinc acetate to the solution of βCD produced intense, doubly charged $(M + Zn)^{2+}$ (base peak), $(M + 2Zn)^{2+}$, and $(M + 3Zn)^{2+}$ ions (Fig. 1*d*). This feature can be used to determine the molecular weights of CDs that have masses higher than the range of the spectrometer. The addition of Me^{2+} induces the formation of doubly charged ions and brings the m/z value down to a measurable range. The presence


+LiCl
(a)
(M+Li)+
1142.0
574.5
1155.0
(M+Na)+
1158.0
(M+K)+
1173.5
(M+Li+LiCl)+
1184.0
Relative intensity (%)
0
25
50
75
100
500
1000
1500
2000
m/z
+RbCl
(b)
87.5
Rb+
85.5
207.0
(M+Rb)+
1220.0
(M+Rb+RbCl)+
1342.0
Relative intensity (%)
0
25
50
75
100
500
1000
1500
2000
m/z
+CsCl
(c)
Cs+
133.0
163.5
301.0
(M+Cs)+
1268.0
(M+Cs+CsCl)+
1436.0
Relative intensity (%)
0
25
50
75
100
500
1000
1500
2000
m/z
+ Zn(Ac)2
(d)
(M+Zn)2+
599.5
(M+2 Zn)2+
631.5
(M+3 Zn)2+
663.5
(M+Zn)+
1199.5
64.5
66.5
68.5
29.7
22.2
14.8
7.4
0
Relative intensity (%)
0
25
50
75
100
500
750
1000
1250
m/z

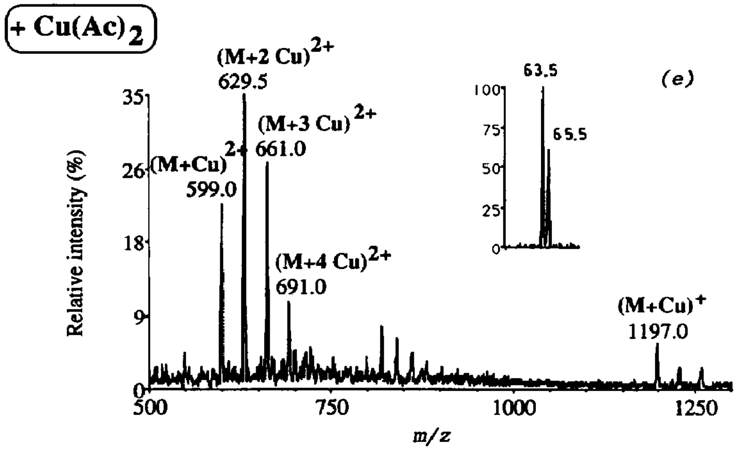


Fig. 1. I.s.-mass spectrum of 100μM βCD (1, mol. wt. 1335.0) in the presence of 100μM (*a*) lithium chloride, (*b*) rubidium chloride, (*c*) caesium chloride, (*d*) zinc acetate, and (*e*) copper(II) acetate.

of copper(II) acetate afforded $(M + Cu)^{2+}$, $(M + 2Cu)^{2+}$, $(M + 3Cu)^{2+}$, and $(M + 4Cu)^{2+}$ ions (Fig. 1*e*). In i.s.-mass spectra, the isotopes of zinc and copper (base peak) could be observed. Besides the doubly charged ions, the ions $(M + Zn)^+$ and $(M + Cu)^+$ were also formed but with low intensity. Especially for zinc, this is an unexpected observation because one of the positive charges must have been "neutralised" in this cluster ion, which implies a reduction of Zn^{2+} to Zn^+.

The occurrence and intensity of all cationised ions with more than one cation, $(M + n\mathrm{Me})^+$ and $(M + n\mathrm{Me})^{2+}$, and also the incorporation of salt $(M + \mathrm{Me} + n\mathrm{MeX})^+$ depended on the final concentration of the salt in the solution of the sample.

Alkylated CDs (**2**–**6**). — Methylated βCDs are sparingly soluble in water, and chloroform–methanol–aqueous 10% formic acid (2:3:1) was used as the solvent. The i.s.-mass spectrum of methylated αCD (**2**) (Fig. 2*a*) contains the ions $(M + H)^+$, $(M + Na)^+$, and $(M + K)^+$ together with such ions as $(M + H + H_2O)^+$ and $(M + H + 2H_2O)^+$. The corresponding doubly charged ions are present also with high intensity (base peak), but there are no higher charged ions. The addition of lithium chloride enhanced the intensity of the doubly charged ions and suppressed fragmentation as well as clustering with other cations such as Na^+ or K^+ (Fig. 2*b*). Therefore, the spectrum is less complex and the peaks can be assigned more easily. The base peak in the i.s.-spectrum when lithium chloride was present was the ion $(M + 2Li)^{2+}$. In addition, $(M + Li + n\mathrm{LiCl})^{2+}$ and similar peaks were observed. The i.s.-mass spectra of methylated α- (**2**) and γ-CD (**4**) were similar to that of the βCD derivative (**3**) (data not shown).

The i.s.-mass spectrum of deuteriomethylated βCD (**5**) contained the ions $(M + H)^+$, $(M + Na)^+$, $(M + K)^+$, and $(M + H + H_2O)^+$. Although the peak for the quasi-molecular ion was abundant, there were numerous peaks in the range 50–250 a.m.u., which could not be assigned unambiguously. These peaks were found in all of the other spectra discussed and are probably fragment peaks clustered with solvent molecules, and thus difficult to interpret.

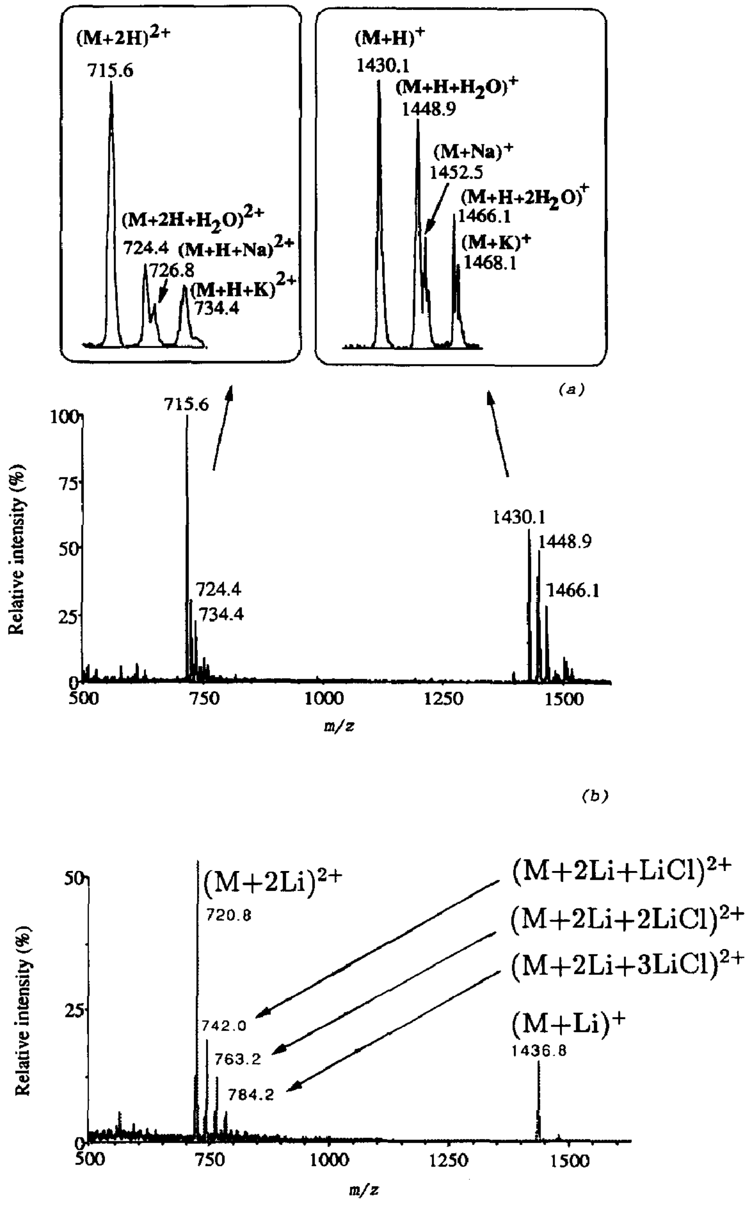


Fig. 2. I.s.-mass spectrum of heptakis(2,3,6-tri-*O*-methyl)-βCD (**3**, mol. wt. 1429.0), (*a*) in the absence of and (*b*) in the presence of lithium chloride

Since the molecular weight (2605) of *n*-pentylated βCD (**6**) exceeds the mass range of the spectrometer used, zinc acetate was added to induce the formation of doubly charged ions. The i.s.-mass spectrum of **6** (Fig. 3) showed βCD with five pentyl groups to be the main product, as indicated by the corresponding $(M + Zn)^{2+}$ and $(M + Na + H)^{2+}$ peaks. The $(M + Na + H)^{2+}$ ions were not observed in the absence of zinc acetate.

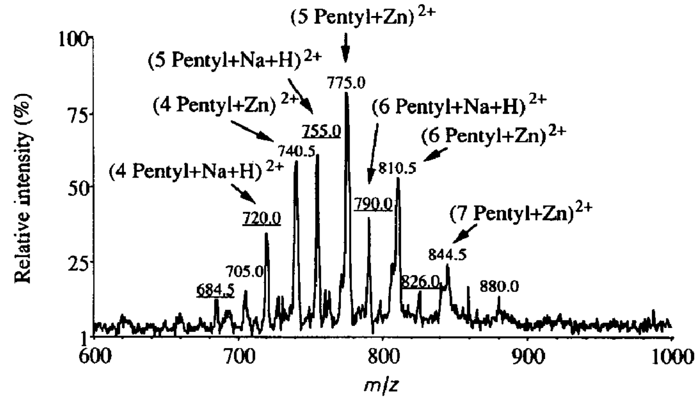


Fig. 3. I.s.-mass spectrum in the presence of zinc acetate of the mixture obtained after *n*-pentylation of *β*-CD; the main product is the penta-*O*-pentyl derivative.

Heptakis(2,6-di-O-methyl)-βCD (**1**). — Hitherto, the analysis and determination of the purity of partially methylated CDs has been difficult. Recently, f.a.b.-m.s. has been used to determine the molecular weights of adducts between partially methylated α- or *β*-CD and organic and metallo-organic compounds[24]. The i.s.-mass spectrum (Fig. 4) of a solution of **1** in chloroform–methanol–aqueous 1% ammonium acetate revealed the expected product (14 methyl groups) but also species with up to five additional methyl groups in the form of $(M + NH_4)^+$ and $(M + Na)^+$ ions. Again, the addition of LiCl simplified the spectrum by producing the corresponding $(M + Li)^+$ and $(M + 2Li)^{2+}$ peaks, and suppressing clusters with other cations. Thus, i.s.-mass spectrometry can be used to determine directly the composition of complex mixtures. The suppression of certain components in mixtures, as is common, for example, in f.a.b.-mass spectrometry, was not observed.

CDs with mixed substituents (**8**–**12**). — Acylation of **7** in order to obtain heptakis-(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-*β*CD (**8**) and heptakis(3-*O*-heptafluorobutanoyl-2,6-di-*O*-methyl)-*β*CD (**9**) yielded mixtures. The i.s.-mass spectra of the mixtures (Figs. 5 and 6), after the addition of lithium chloride, showed equidistant groups of singly and doubly charged quasi-molecular ions. The peaks of one group differed by 7 $[(M + 2Li)^{2+})]$ and 14 a.m.u. $[(M + Li)^+)]$, respectively, corresponding to derivatives that differed in the number of *O*-methyl groups. The difference between the corresponding peaks of two neighbouring groups represents the mass of one trifluoroacetyl or heptafluorobutanoyl moiety, respectively: 96 (singly charged ions of **8**), 196 (singly charged ions of **9**), and 98 a.m.u. (doubly charged ions of **9**). The main products contained 14–16 methyl groups and 2–7 trifluoroacetyl or heptafluorobutanoyl groups.

The i.s.-mass spectrum (Fig. 7) of the *O*-allyl-*O*-methylcyclodextrin **10** shows a range of equidistant $(M + NH_4)^+$ or $(M + H_2O + H)^+$ peaks, the difference in mass reflecting allyl or methyl groups (all positions which were not allylated were methylated). The i.s.-mass spectra of the corresponding pentenyl (**11**) and octenyl (**12**) derivatives yielded similar results (Fig. 7).

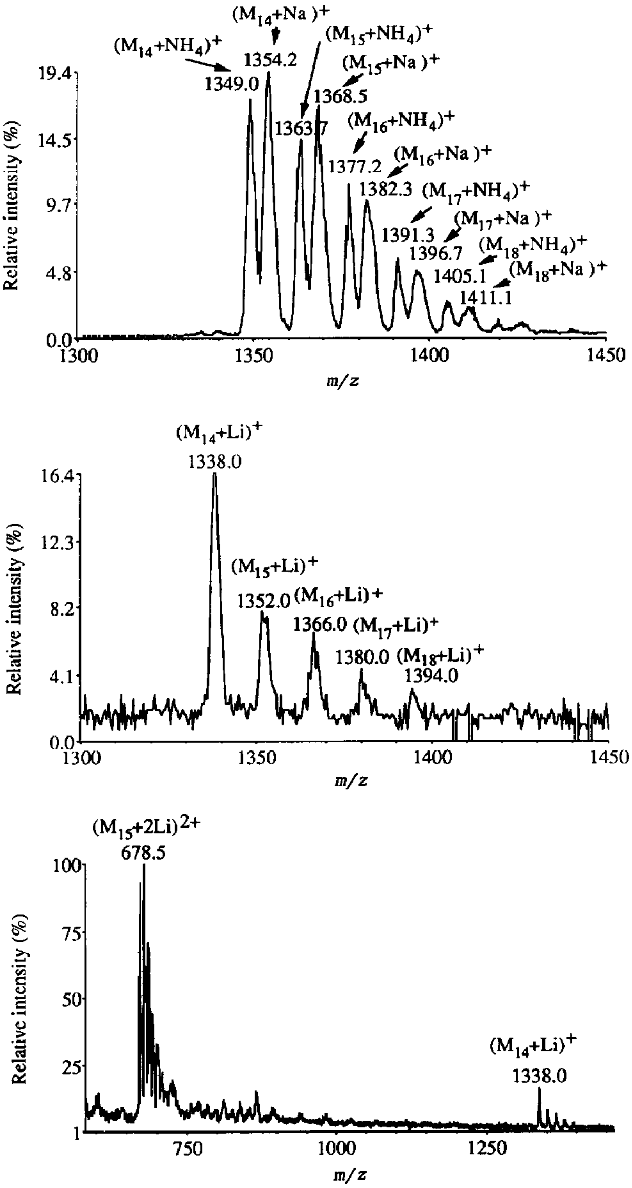


Fig. 4. I.s.-mass spectrum of heptakis(2,6-di-*O*-methyl)-βCD (7, mol. wt. 1331.0), showing a mixture of products containing *a* methyl groups; $[M_a + X]^+$ (for X = Na, NH_4) indicates the corresponding singly charged ion. The addition of LiCl produces doubly charged lithiated ions with high intensity.

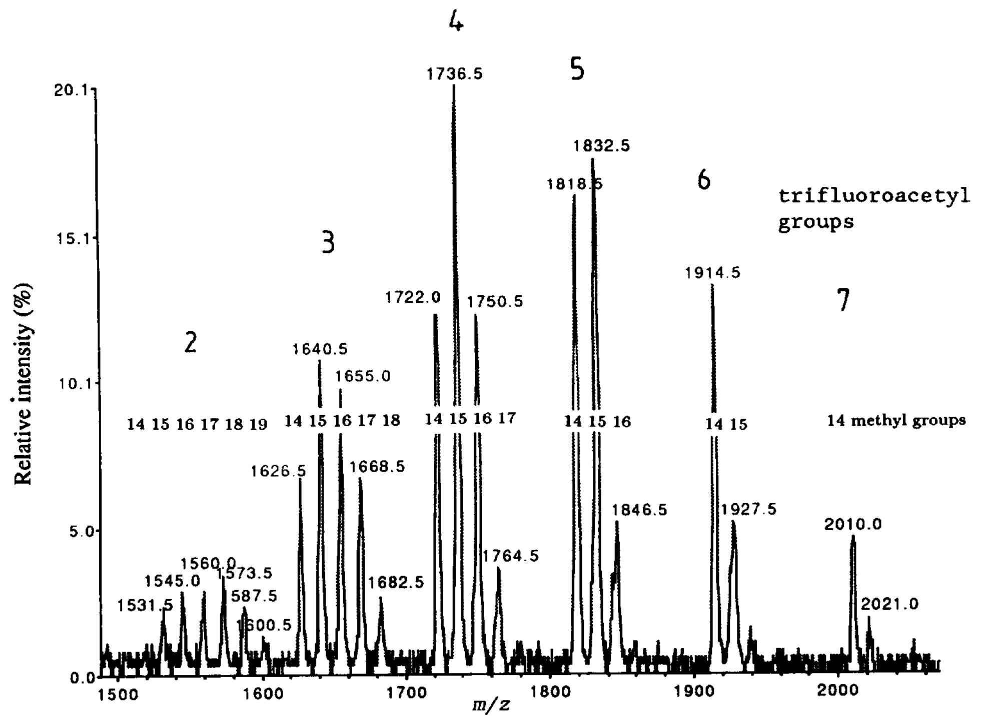


Fig. 5. I.s.-mass spectrum of heptakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-βCD (**8**, mol. wt. 2003.1) in the presence of lithium chloride, showing a mixture of products, each represented by the corresponding singly charged $[M + Li]^+$ ion.

I.s.-tandem mass spectrometry. — Soft ionisation techniques such as ion spray usually produce little or no fragmentation. Additional information on structure, however, can be obtained by i.s.-tandem mass spectrometry. Thus, selected ions are fragmented by collision-induced dissociation with argon in the high-vacuum region of a triple-quadrupole mass spectrometer. The resulting fragmentation may be similar to that obtained by electron-impact ionisation. Moreover, this technique can distinguish between intact molecules and fragments. An example is shown in Fig. 8 for the βCD derivative **9**: the i.s. daughter spectrum of the 2357 a.m.u. peak does not show the adjacent peaks 2342, 2328, 2174 a.m.u., *etc.*, of the parent spectrum, thus proving that the latter peaks do not result from the parent peak by fragmentation. Numerous other daughter spectra gave similar results.

Purity of CD derivatives. — For the methylated CD derivatives **2–5**, the quasimolecular ion expected for the fully methylated product was observed (*cf.* Fig. 2). Incompletely methylated derivatives were absent. The mass spectrum of deuteriomethyl-βCD (**4**) exhibited the predicted higher mass of 63 in comparison to that of **3**. Thus, all compounds were pure fully methylated CDs.

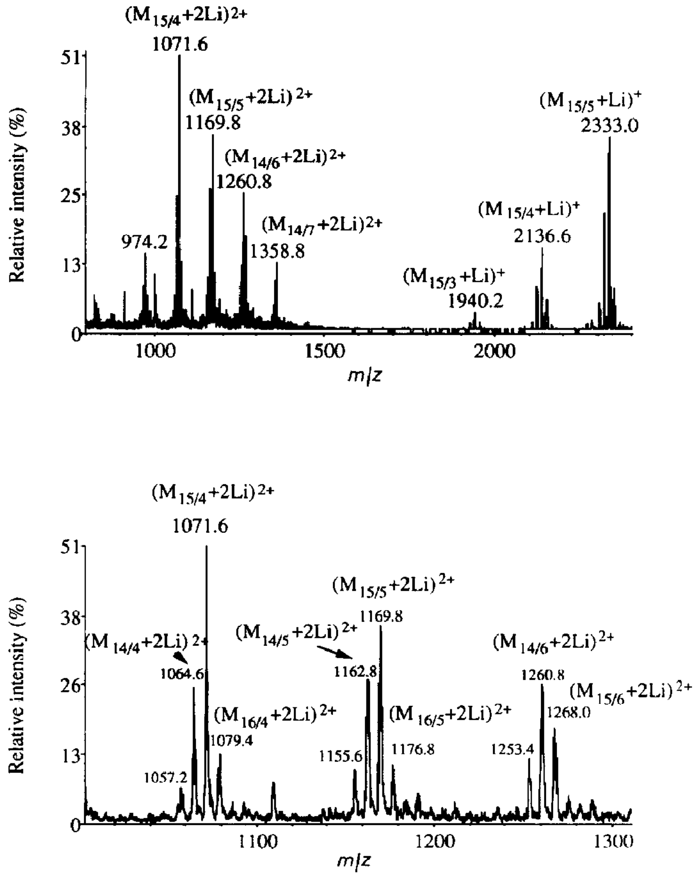


Fig. 6. I.s.-mass spectrum of heptakis(3-*O*-heptafluorobutanoyl-2,6-di-*O*-methyl)-βCD (**9**, mol. wt. 2703.5) in the presence of lithium chloride, showing a mixture of products containing *a* methyl and *b* heptafluorobutanoyl groups; $[M_{a/b} + 2Li]^{2+}$ and $[M_{a/b} + Li]^{+}$ indicate the corresponding doubly and singly charged lithiated ions.

In order to obtain the *n*-pentylated βCD **6**, βCD was alkylated with pentyl bromide. However, the i.s.-mass spectrum (Fig. 3) revealed a mixture of products with 3–7 pentyl groups, with the penta-substituted compound preponderating.

The i.s.-mass spectrum of the regioselectively di-*O*-methylated CD **7** (purified by column chromatography) clearly shows over-methylation (see Fig. 4). Molecular weights associated with under-methylation were absent. The low purity of **7**, also suspected by others[5], is reflected in the 3-perfluoroacyl derivatives **8** and **9**. Indeed, the i.s.-mass spectrum of **8** (see Fig. 5) shows a mass distribution associated with 2–7 trifluoroacetyl groups, with the tetra-acylate preponderating. The remaining 3-positions are either free or are methylated. The mass spectrum of **9** (Fig. 6) is analogous to that of **8**, except that the $(M + 2Li)^{2+}$ peaks have to be evaluated because some of the $(M + Li)^{+}$ peaks are beyond the range of the mass spectrometer used.

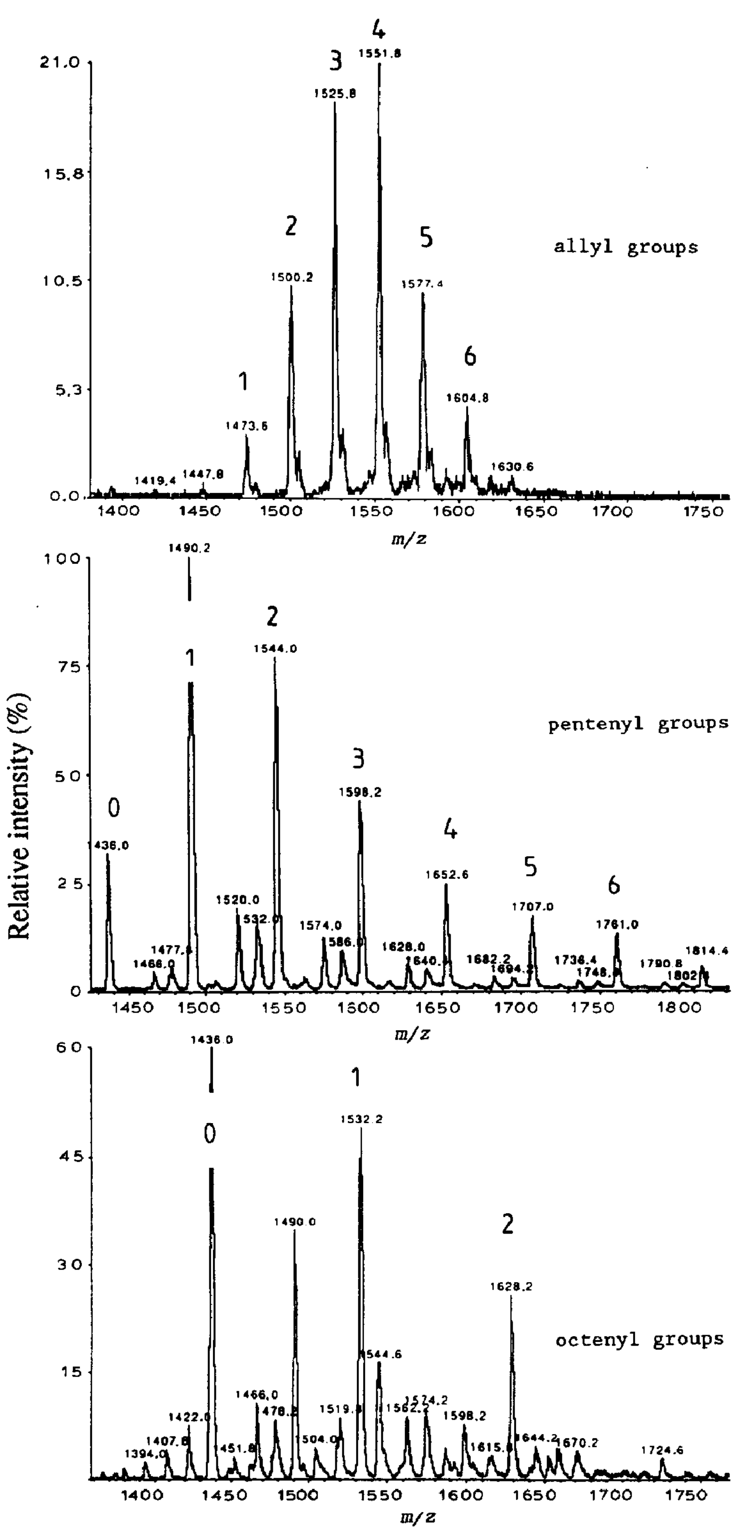


Fig. 7. I.s.-mass spectra of *O*-allyl-*O*-methyl-βCD (**10**), *O*-methyl-*O*-pentenyl-βCD (**11**) and *O*-methyl-*O*-octenyl-βCD (**12**), showing mixtures of products, each represented by the corresponding $[M + NH_4]^+$ or $[M + H_2O + H]^+$ ions.

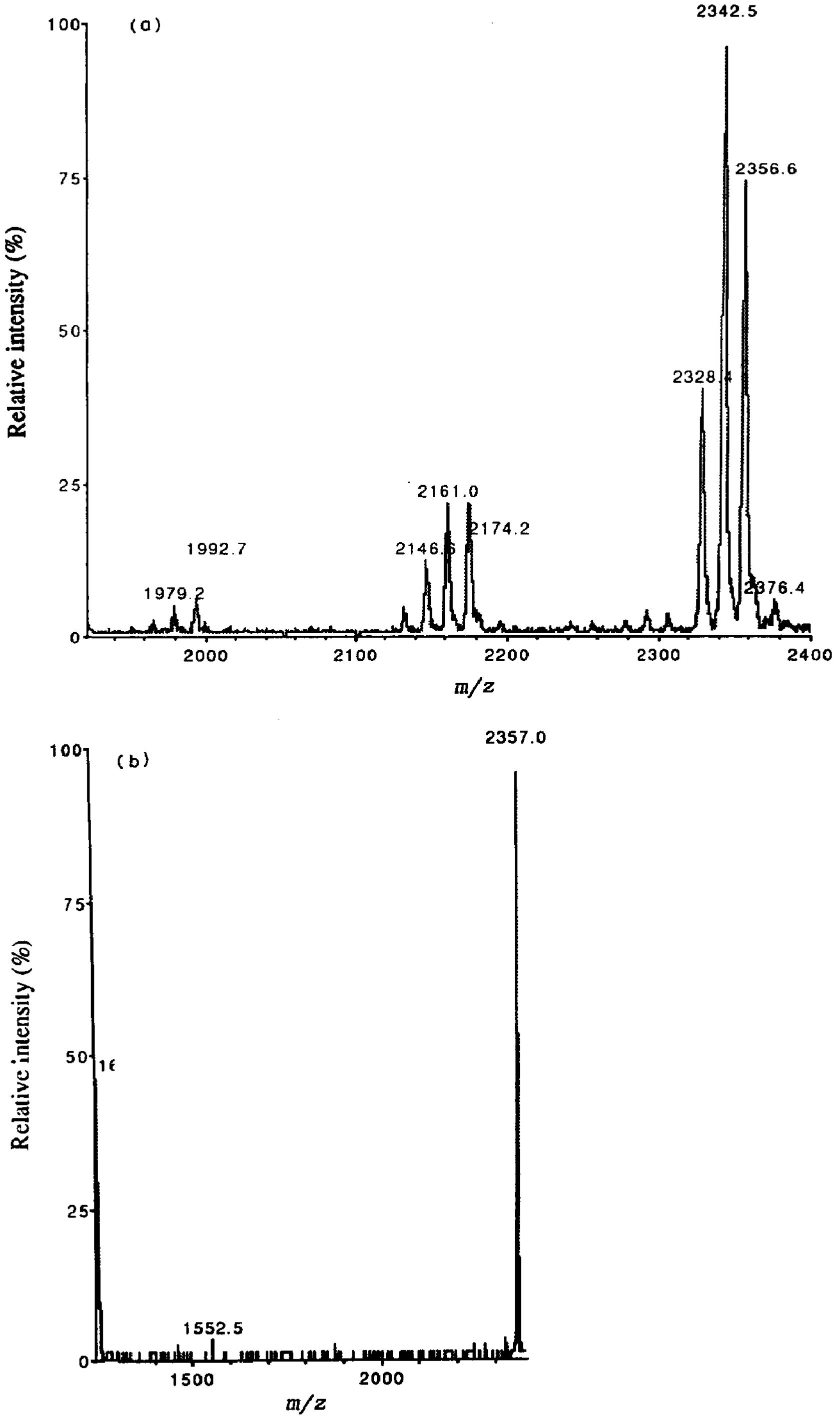


Fig. 8. Tandem mass spectrometry: (*a*) Part of the i.s.-mass spectrum of heptakis(3-*O*-heptafluorobutanoyl-2,6-di-*O*-methyl)-βCD (**9**), showing $[M + NH_4]^+$ or $[M + H_2O + H]^+$ ions, (*b*) i.s.-daughter mass spectrum of the 2356.6 a.m.u. peak in (*a*).

The olefinic derivatives **10**–**12** can be used to bond the CD moiety to a permethyl(hydrido)polysiloxane backbone via platinum-catalysed hydrosilylation[21]. The i.s.-mass spectra (*cf.* Fig. 7) showed that **10** contained 1–7 allyl groups (highest intensity 4), 0–6 pentenyl groups for **11** (highest intensity 2), and only 0–2 octenyl groups for **12** (highest intensity 1).

Thus, i.s.-mass spectrometry is a versatile tool for the analysis of alkylated/acylated CD derivatives, even for complex mixtures.

The addition of lithium chloride or other inorganic salts suppresses fragmentation and clustering with other cations, and facilitates the asignment of peaks and the determination of molecular weights. The addition of zinc acetate favoured the formation of doubly charged molecular ions and facilitates the analysis of CD derivatives with molecular weights that exceed the mass range of the quadrupole instrument.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 323) and Fonds der Chemischen Industrie.

REFERENCES

1 A. P. Bruins, T. R. Covey, and J. D. Henion, *Anal. Chem.*, 59 (1987) 2642–2646
2 T. R. Covey, R. F. Bonner, and B. I. Shushan, *Rapid Commun. Mass Spectrom.*, 2 (1988) 249–253.
3 V. Schurig and H.-P. Nowotny, *J. Chromatogr.*, 441 (1988) 155–163.
4 W. A. König, S. Lutz, P. Mischnick-Lübbecke, B. Brassat, and G. Wenz, *J. Chromatogr.*, 447 (1988) 193–197.
5 D. W. Armstrong, W. Li, C.-D. Chang, and J. Pitha, *Anal. Chem.*, 62 (1990) 914–923.
6 V. Schurig and H.-P. Nowotny, *Angew. Chem. Int. Ed. Engl.*, 29 (1990) 939–957.
7 A. P. Croft and R. A. Bartsch, *Tetrahedron*, 39 (1983) 1417–1474.
8 W. A. König, *Carbohydr. Res.*, 192 (1989) 51–60.
9 W. Li, H. L. Jin, and D. W. Armstrong, *J. Chromatogr.*, 509 (1990) 303–324.
10 G. Wenz, P. Mischnick, R. Krebber, M. Richters, and W. A. König, *J. High Resolut. Chromatogr.*, 13 (1990) 724–728.
11 A. Dell, J. E. Oates, H. R. Morris, and H. Egge, *Int. J. Mass Spectrom. Ion Phys.*, 46 (1983) 415–418.
12 S. K. Sethi, D. L. Smith, and J. A. McCloskey, *Biochem. Biophys. Res. Commun.*, 112 (1983) 126–131.
13 S. Verma, S. C. Pomerantz, S. K. Sethi, and J. A. McCloskey, *Anal. Chem.*, 58 (1986) 2898–2902.
14 K. Harada, M. Suzuki, and H. Kambara, *Org. Mass Spectrom.*, 17 (1982) 386–391.
15 H.-R. Schulten, T. Komori, K. Fujita, A. Shinoda, T. Imoto, and T. Kawasaki, *Carbohydr. Res.*, 107 (1982) 177–186.
16 B. Lindner and U. Seydel, *Anal. Chem.*, 57 (1985) 895–899.
17 D. G. Daves, Jr., W. R. Anderson, Jr., T. D. Lee, H. G. Boettger, and C. E. Giffin, in W. Voelter and D. G. Daves (Eds.), *Biologically Active Principles in Natural Products*, Thieme Verlag, Stuttgart, 1984, pp. 187–194.
18 M. Sakairi and H. Kambara, *Anal. Chem.*, 61 (1989) 1159–1164.
19 H.-P. Nowotny, D. Schmalzing, D. Wistuba, and V. Schurig, *J. High Resolut. Chromatogr.*, 12 (1989) 383–393.
20 V. Schurig, M. Jung, D. Schmalzing, M. Schleimer, J. Duvekot, J. C. Buyten, J. A. Peene, and P. Mussche, *J. High Resolut. Chromatogr.*, 13 (1990) 470–474.
21 V. Schurig, D. Schmalzing, U. Mühleck, M. Jung, M. Schleimer, P. Mussche, J. Duvekot, and J. C. Buyten, *J. High Resolut. Chromatogr.*, 13 (1990) 713–717.
22 V. Schurig and M. Jung, in D. Stevenson and I. D. Wilson (Eds.), *Chiral Separations*, Plenum Press, New York, 1990, pp. 117–133.
23 R. D. Macfarlane and D. F. Torgerson, *Science*, 191 (1976) 920–925.
24 P. R. Ashton, J. F. Stoddart, and R. Zarzycki, *Tetrahedron Lett.*, 29 (1988) 2103–2106.