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LASER-ASSISTED FIELD-DESORPTION MASS SPECTROMETRY OF CYCLOMALTO-HEXAOSE AND -HEPTAOSE AND SOME 6-ALKYLTHIO DERIVATIVES*

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ABSTRACT

The use of laser-assisted field-desorption mass spectrometry for determination of molecular weight, elucidation of structure, and control of purity is demonstrated for cyclomalto-hexaose and -heptaose and their derivatives. Each compound gave an abundant $[M + Na]^+$ ion. The $[Na]^+$ ions originate from traces (~0.1%) of salts in the authentic samples. The fragmentation obtained is structurally highly significant, as the sequential loss of 1–5 sugar subunits is observed. Under these conditions, the elimination of water is negligible, but can be induced by applying higher thermal stress, *e.g.*, using higher laser power. When fragmentation was induced, the cyclic oligosaccharides substituted at positions 6 lost substituted sugar units, thus confirming the synthesis pathway.

INTRODUCTION

Cyclodextrins form 1:1 complexes with a wide variety of molecules and many catalytic processes have been studied[1] using cyclomaltohexaose (α-cyclodextrin, α-CD) or cyclomaltoheptaose (β-cyclodextrin, β-CD) as models for enzyme reactions. In these investigations, a substrate inserted into the cavity of the cyclodextrin may experience selective and accelerated reaction under the hydrophobic conditions of the cavity[2] and/or with the secondary hydroxyl groups (pK_a ~12) on the rim of the cavity[3]. Not only has an appropriate catalytic group been introduced into the cyclodextrin cavity to obtain a more sophisticated model-system of the enzyme[4], but also the inclusion ability has been dramatically enhanced by capping the cavity with a hydrophobic moiety[5,6].

However, the structural determination of these modified cyclodextrins is sometimes troublesome, since they have large molecular weights, carry 18–21 hydroxyl

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*Laser-assisted Field Desorption Mass Spectrometry, Part IV. For Part III, see ref. 14.

groups, and are practically non-volatile. Thus, the application of conventional electron-impact (e.i.) mass spectrometry, even after derivatisation to more-volatile compounds[7], is limited*. Also, these substances give complex n.m.r. and i.r. spectra in which the absorptions of the substituents may be obscured. Likewise, the purity of the cyclodextrins and their derivatives is difficult to quantify. As a consequence, the development of the chemistry of modified cyclodextrins has been retarded.

Field-desorption mass spectrometry (f.d.–m.s.) is now an important method for the investigation of non-volatile and thermally labile substances[8], including determination of the molecular weight of mono- and oligo-saccharides and other sugar-containing natural products[9]. Some characteristic features of the fragmentation of di- and tri-saccharides have been described[10,11]. F.d.-m.s. has been applied to ajugose, an unbranched, plant hexasaccharide, in order to determine its molecular weight; KI was added to promote the formation of $[M + K]^+$ ions[12].

We now report that f.d.-m.s. is very effective for the determination of molecular weight, elucidation of structure, and assessment of the purity of derivatives of α- and β-cyclodextrin. Direct heating of the oligosaccharide samples by an emitter heating current (e.h.c.) caused marked sputtering of the adsorbed material on the emitter surface and only strongly fluctuating f.d. ion-currents could be observed. Indirect heating of the emitter gave much better results. The advantages[13] of laser-assisted f.d.-m.s. include less sample sputtering and less thermal expansion of the emitter, which allows more stable focussing and relatively little thermal degradation of the adsorbed molecules.

EXPERIMENTAL

F.d. spectra (obtained at the University of Bonn) were recorded electrically on a Varian MAT 731 double-focussing instrument, using the Spectro-System MAT 200 for data acquisition and processing. The magnetic scan was performed at 100 m.u./s, and the potential of the f.d. emitter was +8 or +6 kV and that of the cathode −3 or −5 kV. The mass resolution was generally better than 2,000 (10% valley definition). The combined e.i./f.d. ion source was at 50° and 10^{-6} Torr. Methanol–water (1:1) was used for all samples. For desorption of the oligosaccharides, a combination of direct heating (e.h.c.) and indirect heating (laser) was employed. The conditions for "best sample temperature" were determined for each compound (see Figs.). The other experimental details are described in refs. 6 and 13–15, except that an argon ion laser of type INNOVA 90-5 (Coherent, Neu-Isenburg, FRG) was used.

Electrical recording of the f.d. mass spectra (at Kyushu University) was performed with a JEOL D-300 double-focussing mass spectrometer. A scan speed of

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*In e.i.-m.s., perdimethylsilylated derivatives of α-cyclodextrin were reported to give very strong fragmentation with intense ion currents for the fragment ions below *m/z* 207. The base peak of the spectrum was at *m/z* 176. Although all hydroxyl functions were protected as dimethylsilyl derivatives, the relative abundance of the molecular ion at *m/z* 2016 was only 0.14%.

120 or 300 s was used for the mass range *m/z* 300–1300. The potentials for the field anode and slotted cathode plate were +2 and —5 kV, respectively. The mass resolution was 1,500 (10% valley definition). All samples were desorbed by using direct heating, and a small range in e.h.c. (19.9–21 mA) gave relatively weak f.d. ion-currents. The intensities obtained did not allow the use of the JEOL JMA-2000 data system, and the f.d. signals were therefore registered on a paper-chart recorder (SAN-EI Visigraph-5L). All other conditions were as described above.

The α- and β-cyclodextrins were of research-grade purity and commercially available. The preparation of the alkylthio-β-cyclodextrins has been described recently[6].

RESULTS AND DISCUSION

The behaviour of α- and β-cyclodextrins in f.d.-m.s. was studied first. The spectrum of β-cyclodextrin in Fig. 1 shows three characteristic groups of ions.

Attachment of Na^+ and K^+ to the intact molecule leads to cluster ions from which the molecular weight is derived. The $[M + Na]^+$ ion is the base peak of the spectrum and its intensity corresponds to 10,000 counts, at a noise level of 50 counts. To obtain this result, the experimental parameters were chosen in such a manner that minimal thermal stress was applied to the sample (best sample temperature). Stable and intense ion currents were observed by using, for the first time, a combination of an emitter heating current (direct heating) and an argon ion laser (indirect

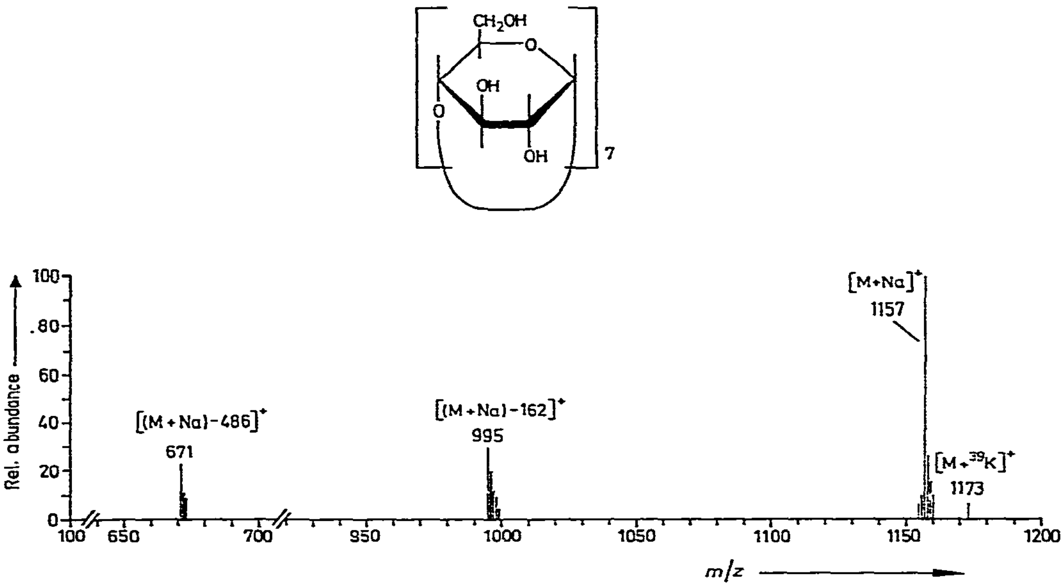

Fig. 1. F.d. mass spectrum of β-cyclodextrin; direct heating (10-mA e.h.c.) combined with indirect, laser heating (30 mW).

heating). For high-resolution instruments and for the desorption of compounds of high molecular weight, the sample supply is improved by this technique. Whereas the heating current transfers thermal energy to the whole sample, the laser beam can be focussed on a small part of the emitter and this additional heating desorbs the f.d. ions very efficiently. The relatively low thermal stress in the f.d. process is reflected by the absence of ions due to the elimination of water. These conditions are desirable when molecular weights are to be determined. Unprotected oligosaccharides containing 5–6 sugar units are borderline cases for f.d.-m.s. using only direct heating. Stable ion currents sufficient for data-system acquisition could not be obtained from α- or β-cyclodextrins using direct heating alone.

Loss of one sugar unit is indicated in Fig. 1 by the $[(M + Na) - 162]^+$ ion at *m/z* 995. Further, in the recorded mass range, *m/z* 100–1200, the loss of two glucosyl units is reflected by the weak ion (5%) at *m/z* 833 for $[(M + Na) - 324]^+$ and the loss of three sugar units by the ion at *m/z* 671. In contrast, the f.d. spectrum of β-CD obtained at an e.h.c. of 20.3 mA gave an $[M + Na]^+$ ion (25%) only. Moreover, an ion at *m/z* 1139 (20%) for $[(M + Na] - H_2O]^+$ was obtained. Nevertheless, all essential signals for the loss of one, two, and three sugar units were found, *m/z* 671 being the base peak. These ions are also accompanied by a prominent series of signals that reflect the loss of water. Thus, the Japanese group showed that f.d.-m.s. of

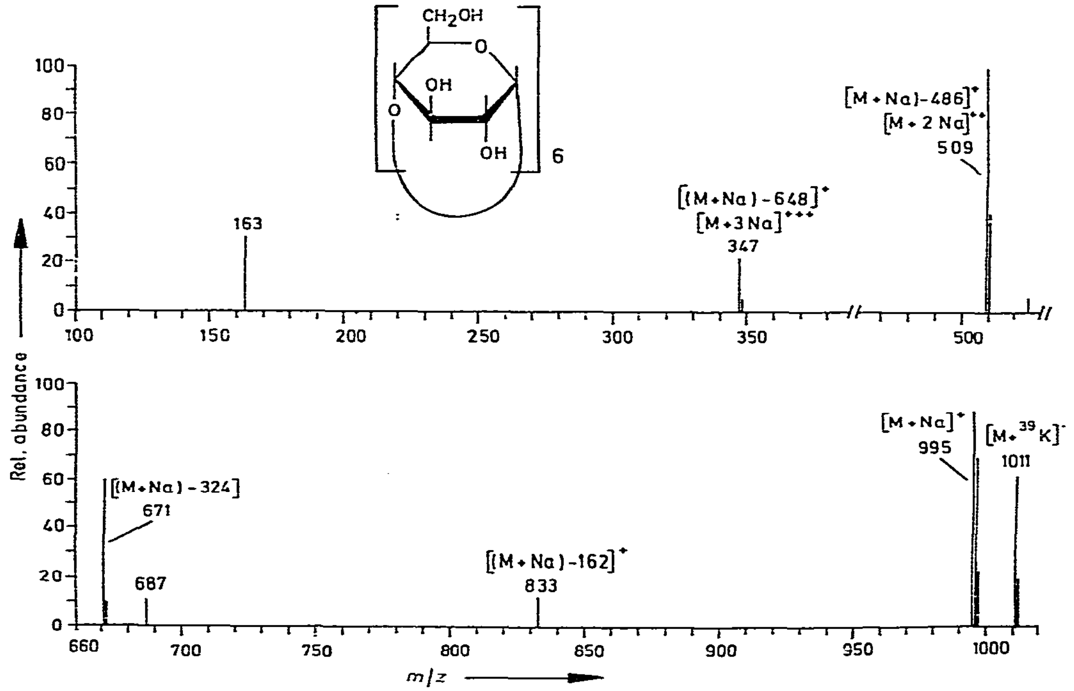

Fig. 2. F.d. mass spectrum of α-cyclodextrin; direct heating (20-mA e.h.c.) combined with indirect, laser heating (20 mW).

cyclodextrins can be performed by using direct heating alone and that essentially the same analytical information is obtained independently. The inter-laboratory reproducibility[16] of the method is satisfying.

After complete decomposition of the organic matrix, the detection and determination of metallic constituents and inorganic salts are possible. Using laser heating, intense ion currents for the alkali and alkaline earth cations were found, often accompanied by salt clusters of sodium or potassium chloride and/or acetate, *etc.* These inorganic impurities, for which f.d.-m.s. is extremely sensitive, are partly responsible for the strong ion-current fluctuations. On the other hand, if the determination of the metal cations is important, it can be achieved on one sample[15].

To explore the feasibility of sequential analysis of oligosaccharides, the f.d. mass spectrum of α-CD was recorded (Fig. 2). Intense ions were found for the cationised, intact molecule at *m/z* 995 (92%) and 1011. The latter cluster ion generated by $[K]^+$ attachment is exceptionally abundant and, together with those at *m/z* 687 and 525, reflects the contamination of the α-CD sample by potassium. Since the loss of 162, 324, 486, and 648 mass-units from the molecular cluster is clearly seen in Fig. 2, this reflects the complete sequence of sugar units in the oligosaccharide. The last glucosyl unit, associated with the ion at *m/z* 163 and produced according to the mechanism described previously[17], contained no sodium or potassium. This finding supports the tentative explanation of the fragmentation of α-CD and its homologues by solvolysis on the emitter surface and ion formation by cationisation. It is assumed that reaction with water leads to ring opening and that the resulting acyclic species is thermally cleaved under high-field conditions. In addition, salt formation occurs, preferentially at HO-2 of the glucosyl residue[3], and contributes to the formation of fragments. This is consistent with the observed relative abundances of the fragment ions, which yield a maximum for the three sugar units at *m/z* 509 (base peak, 37800 counts). However, even at high mass resolution, the exact intensity of this ion cannot be determined, since it is superimposed on the molecular cluster $[M + 2Na]^{2+}$. Also, the $[(M + Na) - 648]^+$ and $[M + 3Na]^{3+}$ ions have the same accurate mass (347.095) and cannot be distinguished. Therefore, in this case, the isotopic pattern was used for the identification of the individual ion species.

Fig. 3 shows the f.d. mass spectra of *S*-neopentyl and *S*-hexyl derivatives of 6-thio-α-CD. These derivatives were desorbed under similar conditions and each gave the $[M + Na]^+$ ion as base peak. Further comparison also shows that the $[M + 2Na]^{2+}$ ions had similar relative abundances. No fragmentation was observed for the neopentyl derivative, but the hexyl analogue gave a weak signal at *m/z* 1239 (6%) which reflected elimination of water, probably due to the slightly higher laser power.

The temperature range in which the intact, cationised molecule can be desorbed is very narrow. This was shown by the Japanese group for the above derivatives. At 21.2-mA e.h.c., the neopentyl derivative gave the following main signals: *m/z* 1243 (86%, $[M + Na]^+$), 1081 (16%, $[(M + Na) - 162]^+$), 919 (base peak, $[(M + Na) - 324]^+$), 757 (74%, $[(M + Na) - 486]^+$), 595 (22%, $[(M + Na) -$

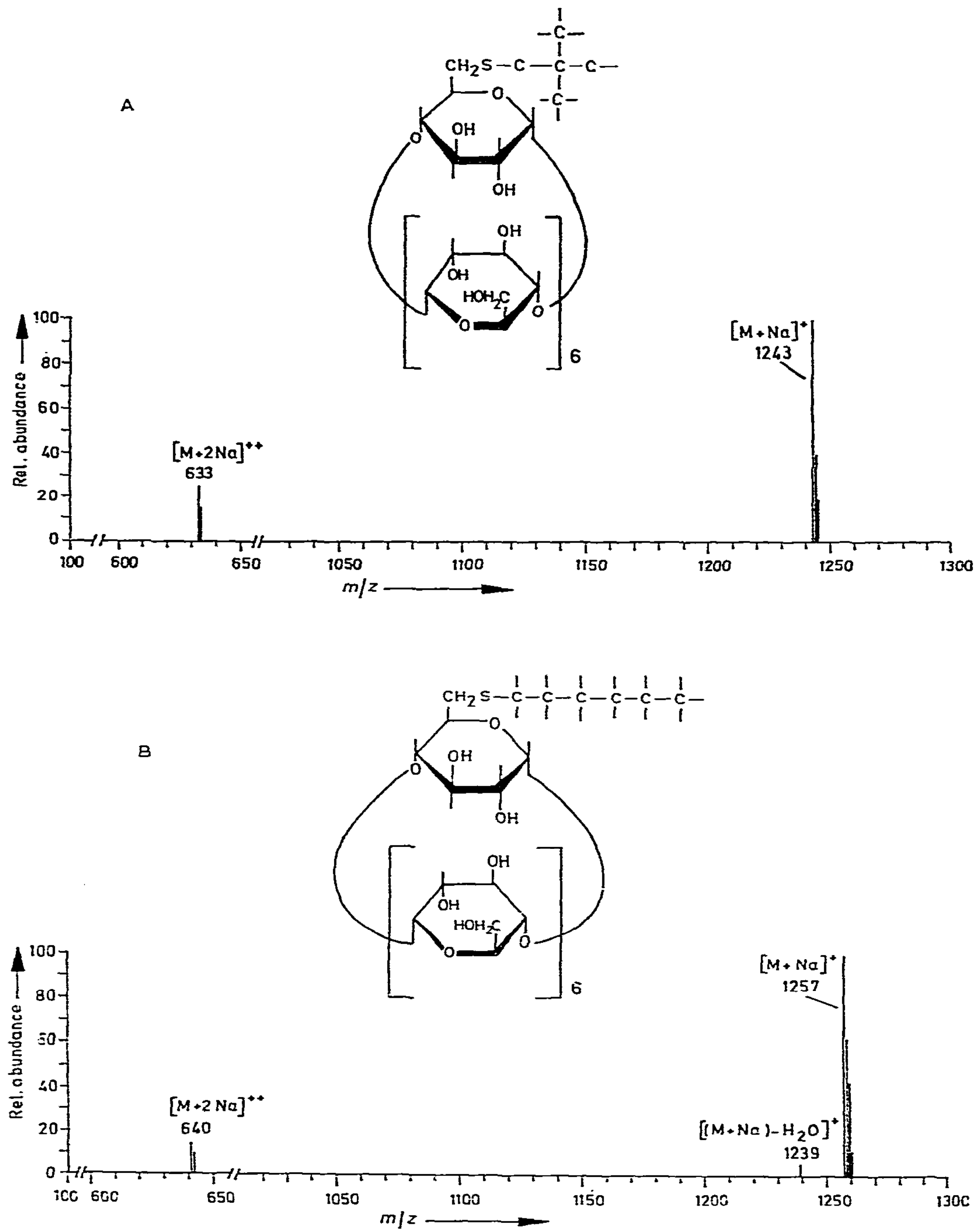

Fig. 3. F.d. spectra of *A*, *S*-neopentyl-6-thio-β-cyclodextrin; 10-mA e.h.c. plus 25-mW laser power (base peak intensity, 34100 counts); *B*, *S*-hexyl-6-thio-β-cyclodextrin; 10-mW e.h.c. plus 60-mW laser power (base peak intensity, 41100 counts).

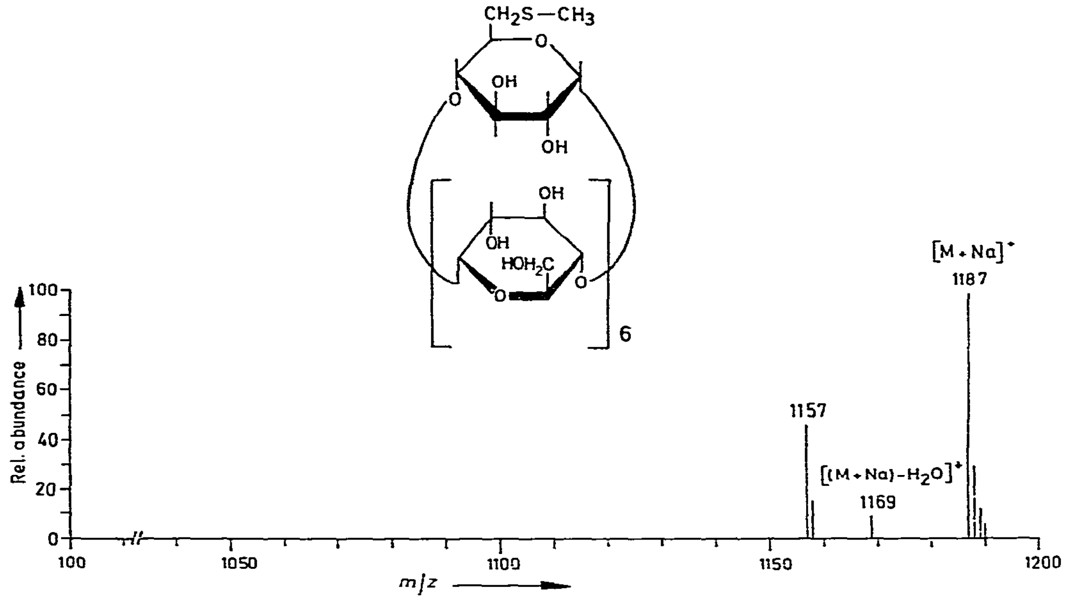

Fig. 4. F.d. mass spectrum of *S*-methyl-6-thio-β-cyclodextrin; 20-mA e.h.c. plus 30–50-mW laser power.

648]$^+$, and 633 [M + 2Na]$^{2+}$. At 21.2 mA, the hexyl derivative gave a very similar f.d. spectrum, although the [M + Na]$^+$ abundance was somewhat lower (39%). Thus, the determination of the molecular weight and the sequence of sugar units is feasible and the data obtained are in good agreement with the types of ions described for α-CD in Fig. 2. Nevertheless, the thermal stress on the molecules is much greater using the e.h.c. alone, and this is indicated by a number of additional ions for which an explanation is difficult as they are produced by thermolysis and intermolecular reactions.

The f.d. spectrum of freshly synthesised *S*-methyl-6-thio-β-CD, obtained using the combination of direct and indirect heating, is shown in Fig. 4. The molecular weight was determined easily, because of the intense [M + Na]$^+$ ion at *m/z* 1187. Raising the laser power from 30 to 50 mW gave the signal at *m/z* 1169, which reflects elimination of water. However, a rather intense ion at *m/z* 1157 was also observed, indicating contamination by the β-CD used in the synthesis. Thus, the potential of f.d.-m.s. in purity control of products of high molecular weight is demonstrated. The method has recently been successfully applied for these purposes to organometallic compounds[18], oligonucleotides[19,20], and lipids[21].

The interpretation of f.d. spectra is not always straightforward. In order to illustrate routine work and to point out some possible pitfalls, the results for a specimen of synthetic *S*-propyl-6-thio-β-CD are given. The original, photographed output is shown in Fig. 5. Since the molecular weight is 1192 (M_1), one would expect, from the results shown and discussed above, to find an intense [M_1 + Na]$^+$ ion at *m/z*

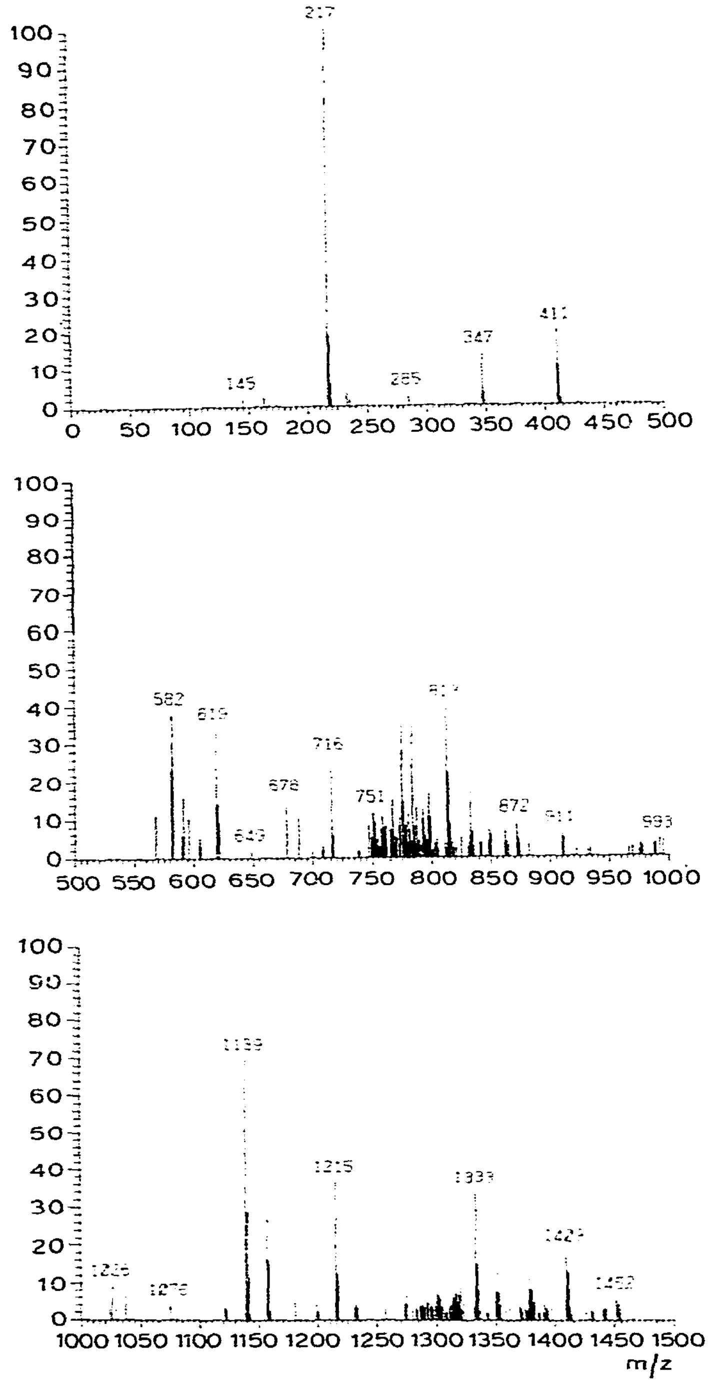

Fig. 5. Original output of the data system for 8 averaged f.d.-mass spectra of *S*-propyl-6-thio-β-cyclodextrin; 10–15-mA e.h.c. plus 20–80-mW laser power.

1215. Although such an ion was recorded with 36% relative abundance, unambiguous identification was difficult because of the complexity of the data. The $[M_1 + 2Na)^{2+}$ ion at *m/z* 619 indicated the presence of the *S*-propyl derivative in the sample. However, at least three more molecules are derived from their sodium clusters, with *m/z* 1139 for $[M_2 + Na]^+$ $(M_2 \hat{=} 1116)$, 1333 for $[M_3 + Na]^+$ $(M_3 \hat{=} 1310)$, and 1409 from which the molecular weight $(M_4 \hat{=} 1386)$ can be calculated. Their doubly charged, cluster ions were found at *m/z* 716, 678, and 581, respectively. A key ion for further interpretation of the f.d. spectrum was at *m/z* 217, due to the cluster (n = 1) containing sodium toluene-*p*-sulphonate, also produced in the synthesis of *S*-propyl-6-thio-*β*-CD. The $[TsONa + Na]^+$ ion series is reflected in the signals at *m/z* 411 (n = 2), 605 (n = 3), and, possibly, 799 (n = 4). However, in the mass ranges *m/z* 750–805 and 1280–1325, uncontrolled outbursts of f.d. ions make reliable assignment of the signals impossible. Taking into account the high intensity of the f.d. ion at *m/z* 217 (117376 counts, base peak), the ion at *m/z* 1409 can be interpreted as M_1 cationised by the salt cluster of TsONa. This means that M_4 is not a new molecular species, but a yet unknown cluster ion of *S*-propyl-6-thio-*β*-CD with the organic salt impurity. Finally, one may speculate that the mass difference between the $[M_1 + Na]^+$ ion at *m/z* 1215 and the signals for M_2 and M_3 at *m/z* 1139 and 1333, respectively, might be due to the loss and transfer of propanethiol.

The above f.d. results demonstrate the superiority of laser heating for the desorption of polar molecules from the emitter surface. Occluded water and chemically bound water, as well as water molecules eliminated by direct heating of the cyclic oligosaccharides, give rise to sputtering (solvent bubbles[22]) and instability of the ion beam. In addition, due to the presence of inorganic contaminants, the salt effect[22] dramatically enhances the fluctuations in the observed ion current. Laser-assisted f.d.-m.s. clearly offers a solution to this problem. Further progress in the analyses of oligosaccharides and their derivatives has been demonstrated recently using high-field magnets[23], integrated electrical recording[24], and the novel concept of physical modification of the emitter surface[25].

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