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FIELD DESORPTION MASS SPECTROMETRY OF NATURAL PRODUCTS—I

STEROID- AND TRITERPENE SAPONINS

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Abstract—Field desorption mass spectra of underivatized oligoglycosides of spirostanol and its congeners and of hederagenin revealed the molecular weight and structural information. The basic predominance of the formation of stable ions of highly polar natural products (with 5–17 free OH functions) is clearly demonstrated. After the extraction and purification procedure from plant material the samples contained inorganic salts in concentrations between 0.01 and 0.1%. These salt impurities, mostly alkali halides, strongly promoted the attachment of alkali cations to the intact molecules *and* their fragments. The ions produced gave analytical information not only for the confirmation of the molecular weight but also for the identity of the aglycone and, to some extent, for the sequence of the sugars in the oligoglycosides. All spectra were recorded on photoplates at a resolution of better than 15000 and enabled the evaluation of the elemental composition of the ions generated. The drawbacks and merits of integrating ion recording using the photoplate for FD investigations of highly polar substances are discussed.

For natural products containing glycosidic sugars studies by electron impact (EI) mass spectrometry (MS) have already been reported on alkyl and aryl hexopyranosides as well as hexofuranosides,[1] aryl glucosides,[2] coumarin glucosides,[3] flavonoid glycosides,[4,5] C-glucosides,[6,7] 1-N-acyl derivatives of glucopyranosylamines,[8–10] 1-phenylflavazol derivatives of oligosaccharides,[11] glycopeptides,[12] nucleosides,[13,14] cardenolides,[15,16] oligosaccharides,[17–25] acetates and methyl esters of spirostanol and related furostanol glycosides[52] and permethylates of triterpene saponins.[62] Field ionization (FI) mass spectra have been published for permethylated oligosaccharides,[26,27] underivatized nucleosides,[28] and naturally occurring glycosides such as somalin[29] and cardenolides.[30,31] With the introduction of field desorption[32] (FD) which allows analyses of underivatized compounds from a solution and avoids evaporation the scope of mass spectrometric investigations has been increased considerably.[33] Free oligosaccharides,[27,34] sugar phosphates,[35] mononucleotides[36] and dinucleoside phosphates,[37,38] antibiotics containing glycosidic aminosugars,[33,39,40] steroid glucuronides,[41] stereoisomeric glycosides[42] and glycosides of the coumarin and flavone series[43] gave high molecular ion intensities and structural information. However, as far as we are aware, FD mass spectra on steroid- and triterpene saponins have not appeared as yet. On the other hand, when glycosides extracted from natural sources, e.g. chlorogenic acid, aesculine, rutin, hesperidin[43] and cascaroside[44] were investigated by FD–MS it became clear that inorganic impurities, mostly alkali halides, play an important role in the formation of ions. Although with conventional analytical techniques only minute amounts of sodium and potassium were detectable the FD spectra showed prominent signals for the attachment of alkali cations (Cat) to the intact molecule (M). This energetically and kinetically favoured process, cationization,[45,46] generates cation complexes $[M+Cat]^+$, which are in general more stable than radical molecular ions or protonated molecules. For molecular weight determination of large, polar substances by FD–MS it was proposed to add on purpose small amounts of cation donors to the sample[46] and recent studies confirmed the usefulness of this procedure.[47,48] However, in our experience with genuine samples extracted from biological material in most cases sufficient amounts of accompanying alkali salts were present to perform cationization. This holds in particular when the ions were produced by emission-controlled FD-MS[49–50] and were recorded by integration on photoplates.[51]

As part of a continuing effort to explore the analytical utility of the FD technique, we have studied the FD mass spectra of free kryptogenin-, spirostanol-, 18-nor-spirostanol-glycosides and hederagenin bisglycosides of high molecular weight after their isolation and purification. We sought answers to the following questions.

(1) What information offers FD–MS of underivatized natural products containing between one and six sugar residues?

(2) What is in practice the influence of alkali salts present in "field samples" of natural products after extraction and purification?

(3) What are the drawbacks and merits of integrating ion recording by the photoplate for FD investigations of highly polar substances from natural sources?

RESULTS AND DISCUSSION

The FD mass spectrum of kryptogenin 3-0-β-D-glucopyranoside (**1**)[53] in Fig. 1 shows the $[M+Na]^+$ ion at *m/e* 615 as the base peak. The corresponding cation complexes of potassium are also pronounced, e.g. *m/e* 631 for $[M+{}^{39}K]^+$ (and additional isotopic peaks). In general, for the samples investigated here the $[M+K]^+$ ions are found with smaller relative intensities than the $[M+Na]^+$ ions. This is consistent with our experience in

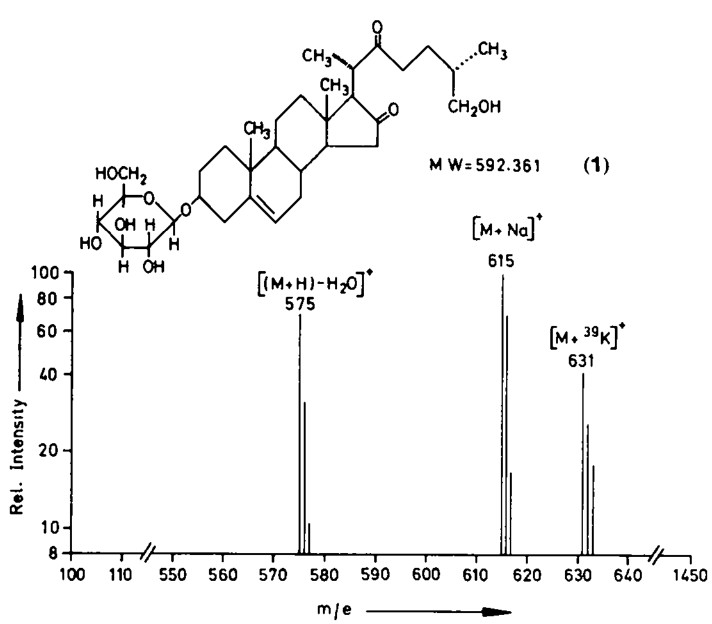

Fig. 1. Photographically recorded FD mass spectrum of 1. Exposure time of the photoplate 10 min. Best anode temperature at 22–24 mA emitter heating current. Alkali concentrations: K < 0.01%, Na = 0.01% (AAS).

the FD analyses of other natural[37,43] and technical[58,59] products. The reason for this is twofold. On one hand, most chemical treatments of natural products are performed with sodium salts. Thus, the concentration of potassium salts in the samples is smaller. On the other hand, recent studies of the cationization process[46] revealed that the signals generated by the attachment of potassium cations to the intact molecule are without exception smaller than those obtained by reaction with lithium or sodium cations under comparable experimental conditions. It appears that the general tendency for cationization decreases in the first group of the periodic system from lithium to cesium.

Since the available commercial instrument is not equipped for emission-controlled FD–MS the emitter heating current for the desorption of 1 was raised linearly from 0 to 50 mA during the exposure time of the photoplate. When in this desorption program all ions were recorded in one track of the photoplate the FD spectrum did not exhibit $[M]^{+\cdot}$ or $[M+H]^{+}$ ions at all but high intensities for the cation clusters of the molecule. The only fragment recorded in the mass range between *m/e* 100 and *m/e* 1450 is due to loss of water from the protonated molecule. This is in sharp contrast to the mass spectra obtained by EI–MS. Because the free glycosides are not amenable to mass spectrometric analysis the permethylates and peracetates were prepared[52] and their EI mass spectra were registered photographically. Numerous fragments were obtained and gave very useful information of the aglycone and the sugar moieties. However, as the relative intensities of the molecular ions are small or unobserved for oligosaccharide glycosides with more than three sugar units, the FD spectra give strong evidence for the confirmation or determination of the molecular weight.

Compound 1 desorbs so smoothly from the FD emitter wire for a time of 10–15 min that an electrically recorded mass spectrum is easily obtained. In comparing the molecular ion region of this spectrum (Fig. 2) with the FD spectrum shown in Fig. 1 two essential facts are illustrated.

First, in contrast to the time consuming processing and data evaluation of the photoplate, the electric detection system rapidly gives straightforward information of the cation complexes. Although the $[M]^{+\cdot}$ and $[M+H]^{+\cdot}$ ions are also not detected the molecular weight of 1 is easily derived.

Second, due to its linear ion response electric recording reflects the true intensity values directly and thus allows an estimation of the isotopic pattern. For the photoplate the tedious procedure to establish the ion response curve for the photographic emulsion has to be worked out. Nevertheless the dynamic range of the photoplate recording is poor, especially for the registration of very low and very high ion intensities (for a comparison see *m/e* 613, 614, 615, 616, 617 in Figs. 1 and 2).

However, in FD–MS, two phenomena can make the electric detection and data aquisition difficult or even impossible. In the first place, field desorption of highly polar substances frequently produces strongly fluctuating ion currents. Secondly, with an increasing number of functional groups in the molecule, the desorption time decreases and may become too short for scanning of a large mass range. As a result, despite the drawbacks mentioned above, the photoplate as a real integrating device for ion recording effectively competes with the electric recording systems. In the following sections this crucial advantage will be demonstrated by the FD mass spectra of natural products carrying up to seventeen hydroxyl functions.

When the FD mass spectrum of a "field sample" of diosgenin 3 - O - α - L - rhamnopyranosyl - (1→4) - [α - L - rhamnopyranosyl - (1→2)] - β - D - glucopyranoside (dioscin, 2)[54] was taken at the best anode temperature[55] the base peak was once again produced by sodium cation attachment to the intact molecule (Fig. 3). The higher stability of the even electron species in comparison with

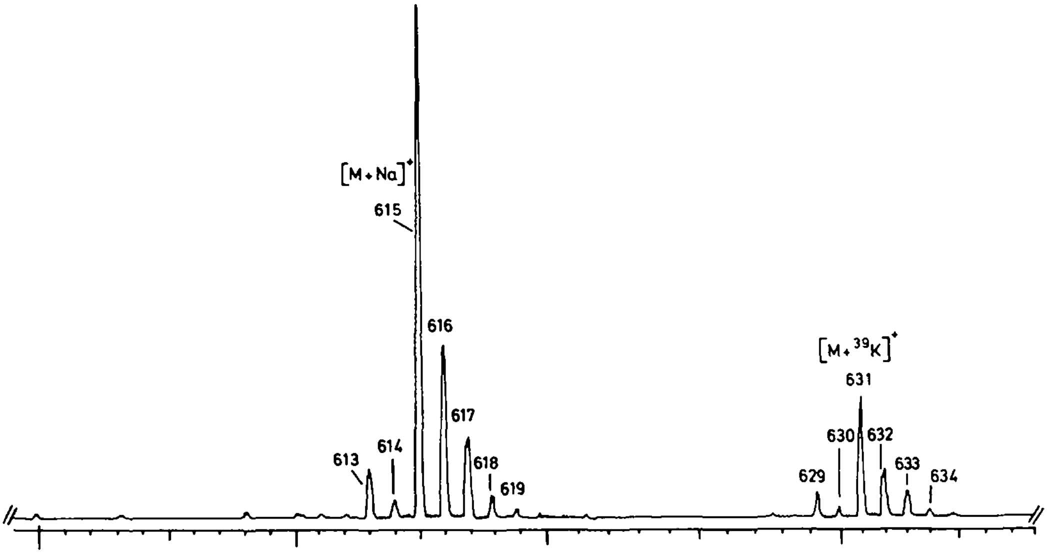

Fig. 2. Electrically recorded FD mass spectrum of 1. Emitter heating current (constant) 24 mA; accelerating voltages +8 kV for the FD emitter and −3.2 kV for the slotted cathode plate. Resolution (10% valley def.) ~2000; amplification factor of the multiplier 1 × 10⁶; output resistance of the multiplier 10⁸ Ω; scan speed 3.5 mu/sec; full scale of the UV recorder 0.1 Volt. Temperature of the ion source 44°.

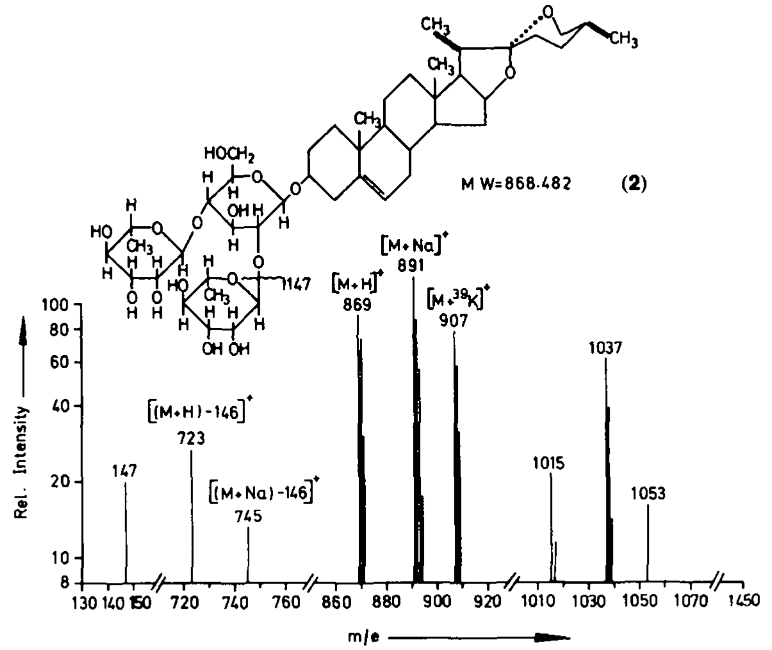

Fig. 3. Photographically recorded FD mass spectrum of a "field sample" of dioscin. Best anode temperature 23–25 mA emitter heating current. Alkali concentrations: $K = 0.13\%$ (flame emission spectroscopy), $Na = 0.09\%$ (AAS).

radical ions is clearly demonstrated. The $[M]^{+\cdot}$ ion is not detected at all but cationization of the molecule with $[H]^+$- and $[K]^+$-ions yields the following as the most intense signals. It is worth noting that the sodium cation complex is more abundant than the protonated molecule. In principle, this phenomena has been explained by charge localization at the alkali cations and is consistent with their high electronegativity.[46] The direct bond fission at the glycosidic oxygen of the terminal monosaccharide unit has been described in detail for the FD-MS of oligosaccharides and appears to be a common feature of the method.[56] All three fragments exhibited, at *m/e* 147 for the rhamnose residue, at *m/e* 723 for the loss of rhamnose from the protonated molecule and at *m/e* 745 for the loss of rhamnose from the sodium cation complex underline the importance of the interglycosidic cleavage in the field desorption of sugars.

The origin of the signals in the higher mass range at *m/e* 1015, 1037 and 1053 could not be explained directly. There are two possible channels for their formation. One less probable pathway would be a yet unknown cluster generation of dioscin itself, e.g. transfer of a rhamnose residue in a surface reaction in the high field. The second more probable explanation, especially in view of the characteristic mass differences 23 and 39, suggests the occurrence of a second high molecular weight compound which is present in the sample at low concentration.

In order to distinguish between these two alternatives an FD mass spectrum of an authentic sample of dioscin was recorded under similar experimental conditions (Fig. 4). Clearly the signals at 1015, 1037 and 1053 are missing in this spectrum and immediately the question after the unknown impurity arose. Another characteristic feature of this FD spectrum of dioscin is the dominating effect of cationization by $[Na]^+$- and $[K]^+$-ions. No molecular ion or fragment ion appears without an alkali cation attached. The cleavage of the terminal rhamnose residue with and without the glycosidic oxygen is strongly pronounced. In addition a doubly charged ion generated by a second cationization of the sodium cation complex of **2** is observed at *m/e* 457.231. The usefulness of these doubly charged ions in the determination of molecular weights by FD–MS has been discussed previously.[56–58]

As an example of a natural product which consists of a free tetrasaccharide and a large spirostanol aglycone the FD mass spectrum of diosgenin 3 - 0 - α - L - rhamnopyranosyl - (1→4) - α - L - rhamnopyranosyl - (1→4) - [α - L - rhamnopyranosyl (1→2)] - β - D glucopyranoside (**3**)[53] is shown in Fig. 5. The $[M+Na]^+$ ion at *m/e* 1037.530 ($C_{51}H_{82}O_{20}Na$) is recorded with saturated blackening of the photoplate and gives by far the most intense signal. The $[M+K]^+$ complexes are of considerably smaller relative abundance (note the logarithmic scale for the relative intensities for photographic recording). The protonated molecule is found just above the threshold level. The fragment ions form two very similar patterns of intensities in comparison with the cation complexes of (**3**).

One, at *m/e* 869 for $[(M+H)\text{-}146]^+$, *m/e* 891 for $[(M+Na)\text{-}146]^+$ and *m/e* 907 for $[(M+{}^{39}K)\text{-}146]^+$ indicates the loss of one of the two equivalent rhamnosyl residues. This direct bond cleavage together with hydrogen transfer occurs as described for (**2**) in Fig. 3. The second fragmentation pathway is similar to that reported for FD of oligosaccharides.[34,56] The ions at *m/e* 723 for $[(M+H)\text{-}292]^+$, *m/e* 745 for $[(M+Na)\text{-}292]^+$ and *m/e* 761 for $[(M+{}^{39}K)\text{-}292]^+$ are produced by the fission in the disaccharidyl residue of the molecule accompanied by hydrogen transfer. The loss of both terminal rhamnose residues is less probable although such a process cannot be excluded and would give isomeric ions as end products. It is remarkable that the stabilisation effect caused by the attachment of alkali cations is easily discerned in both groups of fragments. Fragment ions that have their origin from the $[M+H]^+$ ion are in general enhanced in comparison with the relative intensities of the signals derived from complexes with alkali cations. Other fragments of (**3**) were not recorded in the mass range from *m/e* 100 to *m/e* 720 with the exception of a doubly charged $[M+2Na]^{2+}$ ion at *m/e* 530.260 with 21% relative abundance (cf. **2**, Fig. 4).

The obvious resemblance between the molecular complexes of **3** (*m/e* 1015, *m/e* 1037 and *m/e* 1053 in Fig. 5) and the signals produced by the unidentified component in the "field sample" of **2** (*m/e* 1015, *m/e* 1037 and *m/e* 1053 in Fig. 3) prompted us to carry out accurate mass measurements of these ions in both spectra. These high resolution data revealed that within error limits of ±5 mmu, both peak series have the same accurate mass and most probably, the same elemental composition. For this reason and also because of the very similar distribution of intensities between both ion series (cf. Figs. 3 and 5) it may be assumed that compound **3** is an accompanying impurity in the first sample of **2**. This minute amount of **3** was probably present in the sample, because this compound **2** was derived from **3** by acid hydrolysis. Thus, an illustration of the potential of FD–MS is provided for mixture analysis[59,60] of polar natural products and for control of the purity of an extracted sample.

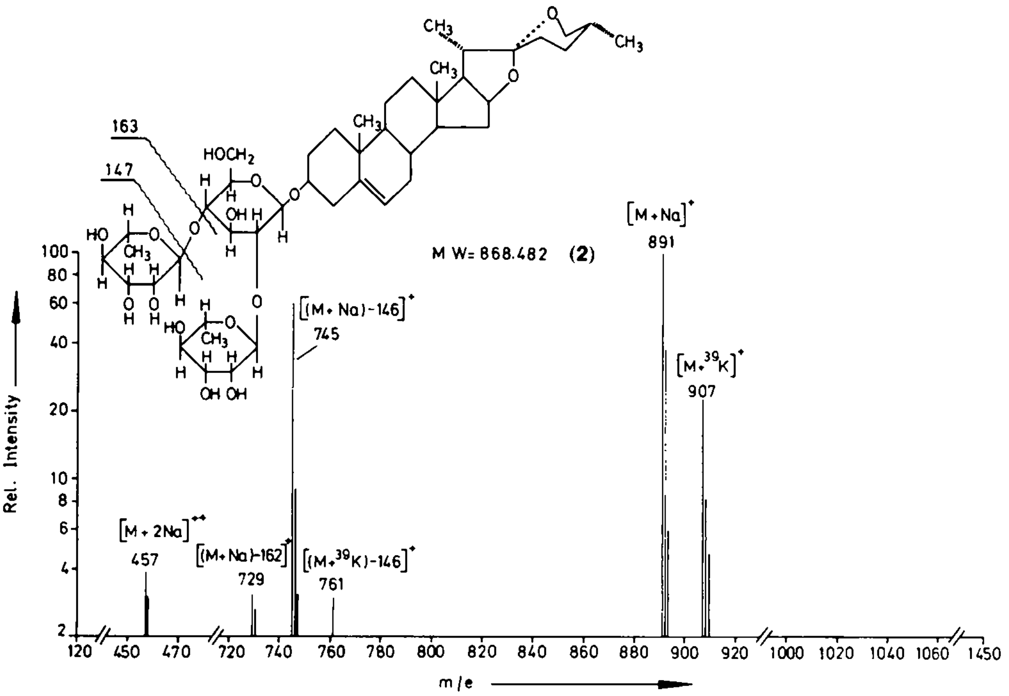

Fig. 4. Photographically recorded FD mass spectrum of an authentic sample of dioscin. Best anode temperature 20–22 mA emitter heating current.

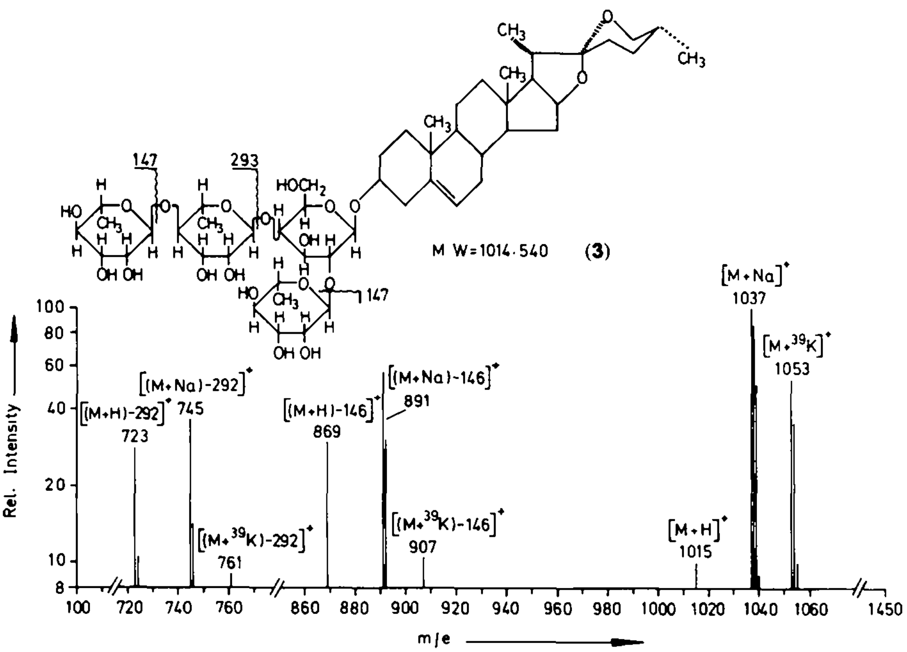

Fig. 5. Photographically recorded FD mass spectrum of 3. Best anode temperature approx. 22 mA emitter heating current. Alkali concentrations: K = 0.04%, Na = 0.1% (AAS). In the lower mass range the complementary sugar fragments at *m/e* 147 and *m/e* 292 are found.

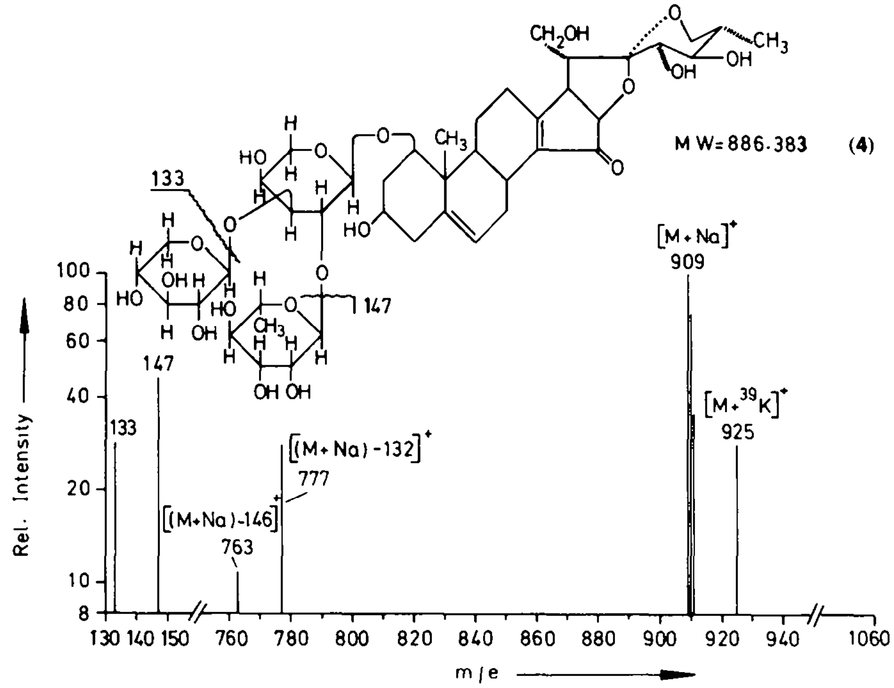

Fig. 6. Photographically recorded FD mass spectrum of 4. Short, fluctuating desorption observed at 25–26 mA emitter heating current. The best anode temperature could not be determined.

With a view to exploring the FD method with compounds of very short desorption times a steroid saponin with eleven free OH functions was examined. The FD mass spectrum of trillenogenin 1 - 0 - α - L - rhamnopyranosyl - (1→2) [-β - D - xylopyranosyl - (1→3)α - L - arabinopyranoside] (4) is displayed in Fig. 6. Once again no $[M]^{+}$ or $[M + H]^{+}$ ions are detected on the photoplate. Above *m/e* 100 only four fragments of 4[61] are found which can be correlated directly to the structure of the saponin. The loss of the xylose residue is indicated by the $[(M + Na)\text{-}132]^{+}$ ion at *m/e* 777, loss of the terminal rhamnosyl rest is marked by the signal at *m/e* 763 for $[(M + Na)\text{-}146]^{+}$. In both cases hydrogen is transferred to the arabinose moiety of 4. In addition characteristic ions at *m/e* 133.050 ($C_5H_9O_4$) for xylose-H and at *m/e* 147.066 ($C_6H_{11}O_4$) for rhamnose-OH are displayed. Very frequently in the FD-MS of sugars and sugar conjugates, this typical cleavage at the glycosidic oxygen is observed which yields two *complementary, charged* parts of the molecule.[56] Together with the knowledge of the molecular weight, which is easily deduced from the cation complexes, the straightforward fission of the molecule into two ionized parts is particularly useful for structural studies. For example, the accurate masses of the peaks at 133 and 147 in Fig. 6 give a clue that two different terminal sugars are present in 4, namely, one pentose and one hexose.

As was observed by Prome and Puzo,[48] the very short desorption time of natural products with more than four sugar units does not allow the electric recording of the whole mass range and only a partial FD spectrum of a hexasaccharide (ajugose) was obtained by juxtaposition of two partial scannings in the *m/e* range of expected peaks of the known compound. We would like to report the first complete mass spectrum of an underivatized triterpene saponin which contains six sugar residues (seventeen OH functions). The FD mass spectrum of 3 - O - β - D - glucopyranosyl - (1→2) - [α - L - rhamnopyranosyl - (1→4)] - α - L - arabinopyranosylhederagenin 28 - O - α - L - rhamnopyranosyl - (1→4) - β - D - glucopyranosyl - (1→6) - β - D - glucopyranoside (5)[62] shown in Fig. 7 exhibits the $[M+Na]^+$ ion at 1405.661 ($C_{65}H_{106}O_{31}Na$) with 35% relative intensity. Due to the integration of all ions generated in the desorption of 5 in one track of the photoplate numerous fragments of direct, high structural significance are registered. Almost all

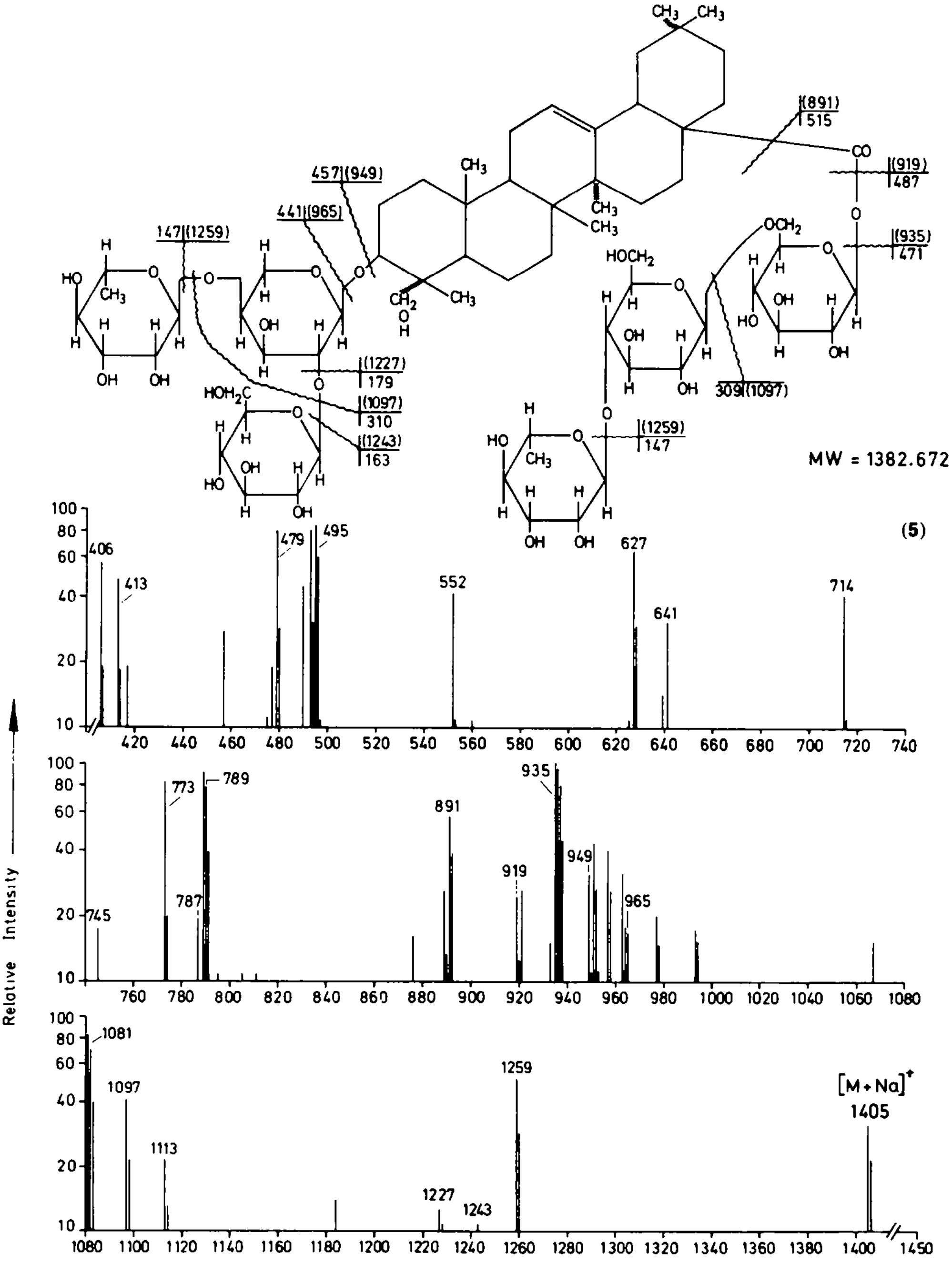

Fig. 7. Photographically recorded FD mass spectrum of 5. Strongly fluctuating desorption above 26 mA emitter heating current.

fragment ions in the higher mass range are produced by cationization.

For the loss of *one* sugar residue, the numbers in brackets in Fig. 7 give the integral mass of the corresponding part of the molecule plus 24 mass units (sodium attachment and transfer of one hydrogen to the vicinal sugar). The cleavage at the terminal glucose residue indicated by *m/e* 1243 and *m/e* 1227 occurs with and without the glycosidic oxygen. The intense signal at *m/e* 1259 reflects the two possibilities for the loss of the rhamnosyl rests both bound in (1→4) position to glucose and arabinose.

For the loss of *two* sugar residues, the numbers in brackets are valid for direct bond cleavage accompanied by sodium attachment and transfer of one hydrogen for the loss of the disaccharide moiety (terminal rhamnopyranosyl (1→4) - β - D - glucopyranosyl residue). Alternatively the signal at *m/e* 1097 might be produced by elimination of the terminal rhamnosyl and the terminal glucosyl rest and transfer of two hydrogens to the arabinose or one to arabinose and one to glucose. Until the present time, no experiment has been performed to distinguish between these different pathways by FD–MS.

For the loss of *three* sugar residues, only the two trisaccharides are considered and the numbers in brackets are valid for sodium attachment and transfer of one hydrogen (as for one sugar). Thus, on one side the ions at *m/e* 935 and *m/e* 919 are tentatively assigned to the cleavage of the rhamnopyranosyl - (1→4) - β - D - glucopyranosyl - (1→6) - β - D - glucopyranosyl moiety from the position 28 of the triterpene. On the other side the peaks at *m/e* 965 and *m/e* 949 indicate the fission in the 3 position of the triterpene and loss of the trisaccharide glucopyranosyl - (1→2) - α - rhamnopyranosyl - (1→4) - α - arabinopyranosyl residue.

Although the interpretations given above are consistent with the evaluated accurate masses more FD investigations of model substances are necessary to establish safe and unambiguous statements in the FD analysis of unknowns. As may be inferred from Fig. 7 there is still a considerable number of intense fragment ions in the mass range below *m/e* 800 which are not easily explained, a whole wealth of structural information in FD–MS has yet to be explored.

EXPERIMENTAL

The FD spectra were produced on a commercial Varian MAT 731 instrument (combined EI/FD ion source) by the *photographic detection* system with vacuum evaporated AgBr photoplates (Ionomet, Waban, Mass., U.S.A.). The resolution obtained was about 15000 (at half peak width), and the average accuracy in the mass determination was ±10 mmu. For accurate mass measurements (in the text the theoretical masses are given) reference masses were taken from the EI and FI mass spectra of tris(perfluoroheptyl)-s-triazine. Field desorption emitters, used in all experiments, were prepared by high temp. activation of $10\,\mu m$ diam. tungsten wires.[45] The distribution and morphology of the microneedles produced were as shown previously.[33] FD emitters with an average length of $30\,\mu m$ for the carbon microneedles were used as standards. The ionization efficiency and the adjustment of the FD emitter were determined by means of *m/e* 58 of acetone in the field ionization mode. All FD spectra were produced at an ion source pressure of 5×10^{-6} Torr. and ion source temp. between 60 and 80°, the accelerating voltages were +6 kV for the field anode and between −2 and −4 kV for the slotted cathode plate. MeOH was used as solvent for all compounds. In general, 1×10^{-6} g was applied as sample to the standard emitter via the syringe technique.[33]

Compound 3 and 4 were isolated from *Trillium kamtschaticum* Pall,[53,61] compound 2 was found in *Dioscerea tokoro* and *Paris polyphylla*[54] and compound 5 in *Akebia quinata* Decne.[62] In those cases where a sufficient amount of sample of the natural products was available (**1**, **2**, **3**) the sodium and potassium concentrations (in weight %) were determined by atomic absorption spectroscopy (AAS) and listed in the Figure Captions.

Summary. In conclusion it can be said that in field desorption mass spectrometry at present no hard and fast rules can be offered on how to interpret an FD mass spectrum of an unknown natural product. Interpretation of FD data is still much of an art and a considerable amount of experience is required to obtain optimum results. However, the principal resemblance between the well established mechanism of glycosidic bond cleavage by acidic solvolysis in solution chemistry and fragment formation under FD conditions could serve as a basis for interpretation in future FD investigations. Moreover, a few general features of the method have been given: cationization by alkali salts, fragment formation by direct bond cleavages and the use of doubly charged ions for confirmation of the molecular weight. Thus, FD-MS complements the results of other analytical techniques on natural products effectively.

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