Field Desorption Mass Spectrometry of Physiologically Active Steroid- and Dammarane-Saponins*

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The use of field desorption mass spectrometry is demonstrated not only for molecular weight determination but also for detailed structural analysis of large, underivatized natural products. Owing to cationization by small alkali salt impurities, saponins carrying one to four sugar units gave abundant quasimolecular ions. In addition, the observed sequence-specific fragment ions reflected the complete sequence of the sugar units in the oligoglycosides. This fragmentation of the oligosaccharidic moiety of the natural products was interpreted by analogy with acidic solvolysis, a mechanism well established in solution chemistry. Of particular interest was a first comparison of the field desorption mass spectra obtained from two different commercially available instruments operated by different groups in order to give an estimate of interlaboratory reproducibility in field desorption mass spectrometry.

Introduction

From the leaves of *Lycopersicon pimpinellifolium* the fungistatic agent tomatine has been isolated and crystallized [1] and its structure accurately characterized as tomatidine 3-O-β-lycotetraoside [2]. It has also been reported [3] that under natural conditions tomatine may act as an insect repellent rather than as toxin. Ginseng, the root of *Panax ginseng* C. A. Meyer, is one of the most well-known Chinese drugs. It contains characteristically a number of dammarane saponins [4, 5] which are classified into 2 groups possessing either 20-S-protopanaxadiol or 20-S-protonanaxatriol as the sapogenins. Preliminary, pharmacological studies of a protopanaxadiol group saponin have shown this substance to be a central nervous system depressing agent, whereas a protopanaxatriol group saponin has been revealed to be an anti-fatigue and a central nervous system activating agent [6].

Electron impact (EI)-mass spectrometry (MS) has been shown to be a very useful method [7–10] for identification, determination of purity, and structural evaluation of saponins. However, for mass spectrometric investigation volatile derivatives have to be produced and often their fragmentation is complicated because of the large and complex structure of the naturally occuring saponins. Moreover, saponins with more than four sugar do not give molecular ions even when derivatized. In continuation of our recent reports of field desorption (FD)-MS of natural products [11–14] here the results are reported of the mass spectrometric studies of physiologically active and structurally typical steroid- and dammarane-saponins. In particular, the fission of terminal sugar units and the sequence-specific cleavages in the oligoglycosidic chain(s) have been evaluated and compared with data from solution chemistry. Further, in order to achieve a better understanding of the general fragmentation pattern of this class of compound gitogenin- [15] and diosgenin- [16] oligoglycosides were recorded by FD-MS as reference substances. The results obtained give a clear demonstration that FD-MS is not only suitable

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for molecular weight determination but is a powerful tool for structural investigation of these underivatized oligoglycosides.

As the free steroid- and dammarane-saponins were studied by two different analytical groups and with different commercially available instruments it was of interest to investigate whether a preliminary, qualitative comparison of the FD results was possible and obtain for the first time information about interlaboratory reproducibility.

Methods

JEOL D-300 instrument, Kyushu University

The FD spectra were produced on a commercial JEOL D-300 instrument (combined EI/FD ion source). All spectra were recorded electrically (scan speed: 120 s or 300 s/full range). The resolution obtained was $R = 1200$ (10% valley definition) and the average accuracy in the mass determination was ± 0.3 mass unit (m. u.). For accurate mass measurements reference masses were taken from the EI mass spectra of perfluorokerosene. Field desorption emitters, used in all experiments, were prepared by high temperature activation of 10 µm diam. tungsten wires. FD emitters with an average length of 30 µm for the carbon microneedles were used as standards. The ionization efficiency and the adjustment of the FD emitter were determined by means of *m/z* 58 of acetone in the field ionization mode. All FD spectra were produced at an ion source pressure of 3×10^{-7} Torr. and ion source temp. between 60 °C and 70 °C, the ion source potentials were + 3 or + 2 kV for the field anode and − 5 kV for the slotted cathode plate. The samples were desorbed by direct heating (emitter heating current) without emission control. MeOH was used as solvent for all compounds. In general, 1×10^{-5} *g* was applied as sample to the standard emitter via the syringe technique. The substances **2, 3, 7, 8,** and **9** were recorded by the D-300 instrument.

Varian MAT 731 instrument, University of Bonn

The same sample amount, same solvent, and the same type of FD emitter was used including the procedure for control of the ionization efficiency. Similar ion source pressure and ion source temperature were maintained as described above. The obtained mass resolution was higher ($R = 2000$, 10% valley definition) which is partly due to the higher ion source potentials of + 6 kV/− 4 kV employed for the Varian instrument. All FD spectra were recorded electrically (scantimes between 4 and 8 s/decade). Data processing was performed by the Spectrosystem MAT 200. Alternatively, the signals were accumulated by a multichannel analyzer (Varian CAT-1024) which was triggered by the cyclic magnetic scan of the mass spectrometer. An example of an original plot of the multichannel analyzer output is shown in Fig. 2.

Results and Discussion

Our previous FD investigations of oligoglycosidic natural products indicated that the process responsible for the cleavage of sugar units at the glycosidic oxygen could be explained by analogy with the well established mechanism of acidic hydrolysis in solution chemistry. Proton transfer reactions in the adsorbed sample on the surface of the FD emitter lead, for instance, to a preferential loss of terminal α-rhamnose in comparison with aglycone-linked β-glucose [12]. In order to evaluate the more general validity of this concept the FD mass spectrum of tomatine (**1**) was recorded (Fig. 1). The characteristic signals obtained can be described as follows:

1. The FD spectrum of **1** shows the $[M+Na]^+$ ion at *m/z* 1056 as the base peak. The corresponding cation complexes of potassium are not observed. The $[M+H]^+$ ion is recorded with 86% rel. abundance.

2. In the FD-spectra of steroid tri- and tetra-glycosides [11, 12] the glucosidic bond cleavage at C-3 of the aglycone was not observed. Consistently also the FD-spectrum of **1** did not show the galactosidic bond cleavage at C-3 of the aglycone.

3. In general, β-D-xylosides are hydrolyzed about 4.8 times faster than β-D-glucosides in solution chemistry [17]. In the FD spectrum of **1** which is averaged from 5 consecutive magnetic scans in the mass range from *m/z* 100 to *m/z* 1100 the loss of the terminal xylose unit ($C_5H_9O_4$, 133 m. u.) leads to the $[(M+Na)-132]^+$ ion at *m/z* 924, and the $[(M+H)-132]^+$ ion at *m/z* 902. Loss of the glucose unit ($C_6H_{11}O_5$, 163 m. u.) gives the $[(M+Na)-162]^+$ ion at *m/z* 894 and $[(M+H)-162]^+$ ion at *m/z* 872, respectively. In this case, cleavage of the terminal xylopyranosyl bond does indeed give the more intense fragment ions than that of the glucopyranosyl bond.

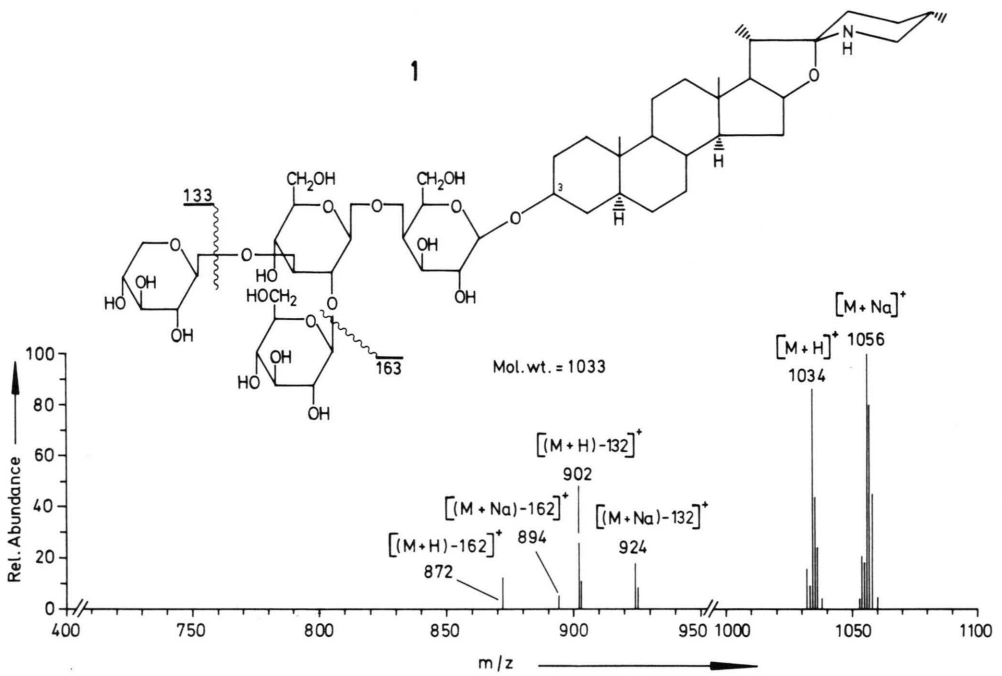


Fig. 1. FD mass spectrum of **1**. Electrical recording (Methods, B), between 30 and 35 mA emitter heating current using the Varian MAT 200 data system; detection threshold 500 counts. The ratio for the loss of glucose : xylose is about 1 : 3.6.

However, due to the relatively short time for the magnetic scan covering the whole mass range, the small number of spectra which were recorded and averaged by the data system and the narrow interval of 30 to 35 mA emitter heating current (close to the best anode temperature (BAT)), only a rough estimate of the prevailing cleavage of the terminal *β*-D-xylose can be made. Much better and more reliable results are achieved using a multichannel analyzer (mca) in combination with the FD mass spectrometer.

The principal advantages of this combination for direct isotope determination of large natural products have been reported recently [13, 14, 18]. Furthermore, quantitation of endogenous [19] and exogenous [20] compounds from physiological fluids, pesticides in HPLC-extracts [21, 22] and ultratraces of metals in high purity solvents [23], human body fluids [24], and heart [25] and brain [26, 42] tissues has been successfully demonstrated using the mca and has widened the scope of analytical applications of FD considerably.

This investigation reports the first results in estimating the relative intensities of the ion currents of two sequence-specific fragment ions in FD. These are produced by the elimination of terminal xylose at *m/z* 924 and terminal glucose at *m/z* 894 from the high molecular weight natural product **1**.

Using repetitive magnetic scans in the mass range from *m/z* 890 to *m/z* 931 a sample of **1** was completely desorbed and the FD ions were recorded in combination with a mca. As shown in the original plot of the mca output (Fig. 2) the long sampling time for each FD peak, small mass range and large number of scans give a clear, reproducible and easily evaluated picture for the loss of terminal sugars. When the ratio of the peak heights of *m/z* 924 and *m/z* 894 was calculated the results of these measurements revealed that in FD-MS for the experimental conditions described the loss of terminal *β*-D-xylose is about 2.8 times more intense than the loss of terminal *β*-D-glucose. Although this ratio is smaller than that for solution chemistry there is still a good agreement with the acidic solvolysis on the emitter surface. No doubt, in analyzing unknown oligoglycosides this analogy would be very helpful. The question arises, however, whether this phenomenon is observed also for other species of sugars.

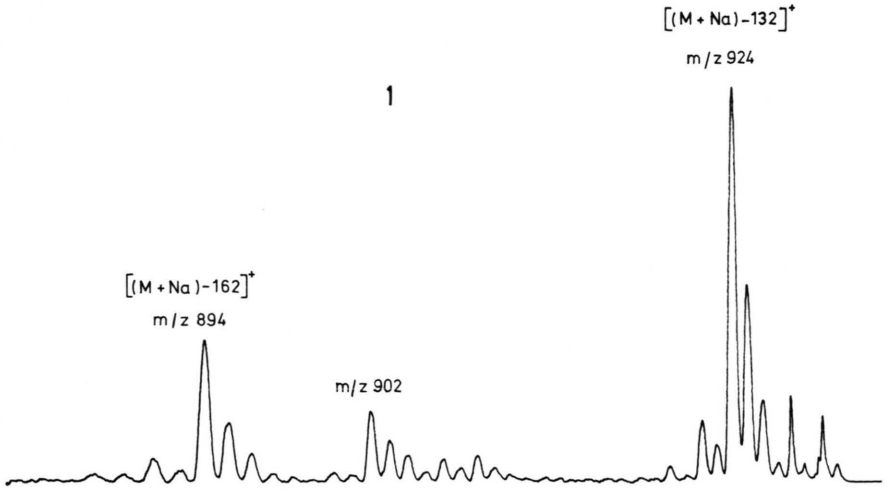


Fig. 2. Original plot of the mca output for the FD ions of **1** in the mass range *m/z* 890 to *m/z* 931. 30 scans were accumulated in the 2^{10} range at a scan speed of 1 m. u./s. No detection threshold was set because it is not required for the registration of FD ions by mca. The ratio for the loss of glucose : xylose was found to be 1 : 2.8.

For trillenogenin 1-O-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)-α-L-arabinopyranoside] it has been reported that the characteristic $[(M + Na)-132]^+$ ion for the loss of terminal β-D-xylose is more abundant in the FD mass spectrum than the $[(M + Na)-146]^+$ ion which indicates the cleavage of terminal α-L-rhamnose [11]. This obviously contradicts the general experience of acidic solvolysis in solution chemistry where α-L-rhamnosides are hydrolyzed about 2.1 times faster than β-D-xylosides [17]. Moreover, since this FD mass spectrum was recorded photographically, the arguments mentioned above for electric registration in connection with a data system do not hold because the photoplate can be regarded as a means for optimal integration in recording FD ions. In order to clarify this point we recorded the sequence-specific ions with the mca and found that at the beginning of the desorption process, when the first 15 scans were accumulated, the ratio was approximately 1 : 1. Later, at emitter heating currents above 28 mA the signal for the loss of the rhamnosyl rest gradually became predominant. It has to be considered, therefore, that the analogy between solution and surface chemistry may only be used as an auxiliary aid for the qualitative interpretation of FD spectra and that the investigation of a wide variety of different model substances is necessary for the correct assignment of FD ions from glycosides of yet unknown structure. On the other hand, it is noteworthy that in both cases, for compound **1** and the previously studied trillogenintriglycoside [11] the loss of the terminal xylopyranosyl rest was slightly more pronounced than the loss of the hexosyl rest for glucose and galactose.

In order to evaluate further the relation between structure and fragmentation pattern for oligoglycosides in FD-MS, the binding position of the sugar should also be considered. There are two possibilities: either a bond to a neighbouring sugar or a bond to the aglycone. In the following, the first of these is investigated. The cleavage of terminal β-D-glucose linked to the neighbouring glucose unit in 3′-position was studied by recording the FD spectrum of diosgenin 3-O-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (= gracillin), **2**. As shown in Fig. 3, electrical registration of the mass range from *m/z* 100 to *m/z* 1000 and averaging of 5 spectra using the data system gives the $[(M + Na)-146]^+$ ion at *m/z* 761 with 39% relative abundance. This signal, significant for the loss of the terminal α-L-rhamnosyl rest is the most abundant sequence-specific fragment ion. Loss of the terminal β-D-glucosyl rest is found with 25% relative abundance of the $[(M + Na)-162]^+$ ion at *m/z* 745. In addition, a weaker signal (14% relative abundance) is recorded at *m/z* 599 for the loss of both terminal sugars. Since in solution chemistry α-L-rhamnosides are hydrolyzed about 10 times faster than β-D-glucosides [17] the FD result is, at least qualitatively, in

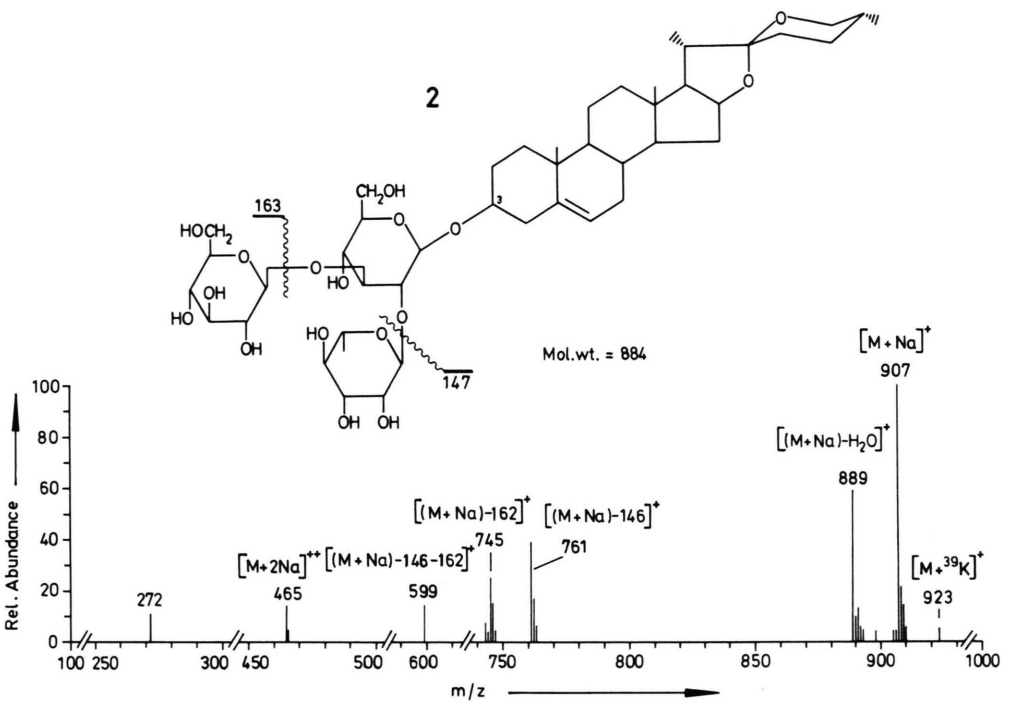


Fig. 3. FD mass spectrum of **2** obtained as described in Fig. 1. When the ratio of the intensities of m/z 761 : m/z 745 for the loss of rhamnose and glucose was determined by the mca at emitter heating currents from 25 to 28 mA this ratio was approximately 1 : 1 (17 scans, range 2^9). At higher emitter heating currents the loss of terminal glucose became more predominant.

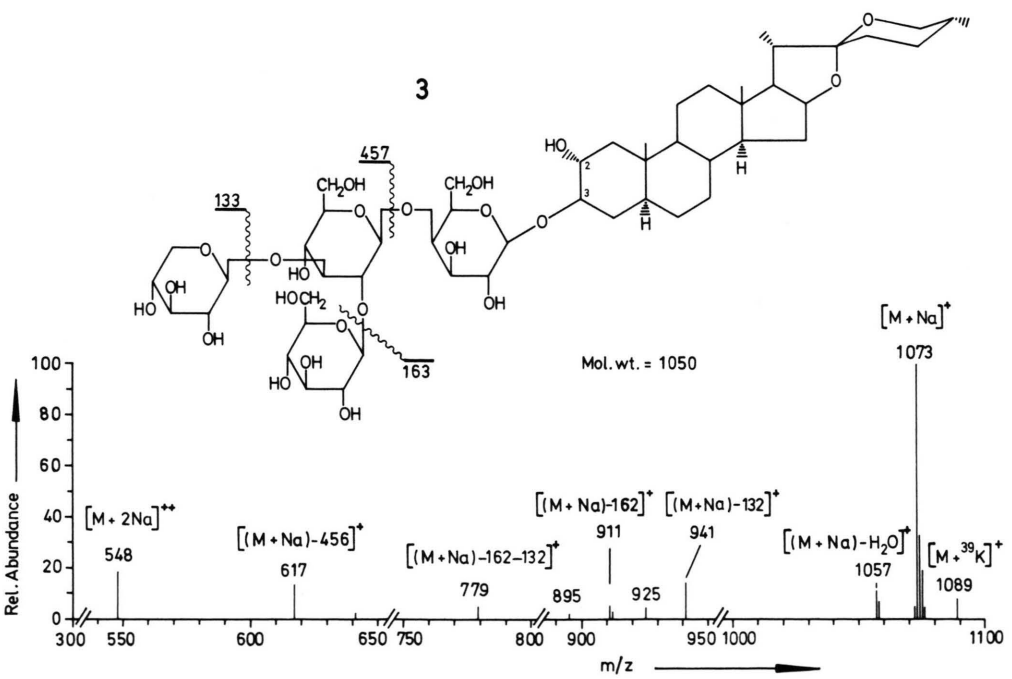


Fig. 4. FD mass spectrum of **3** obtained at 30 mA emitter heating current as described for **1.**

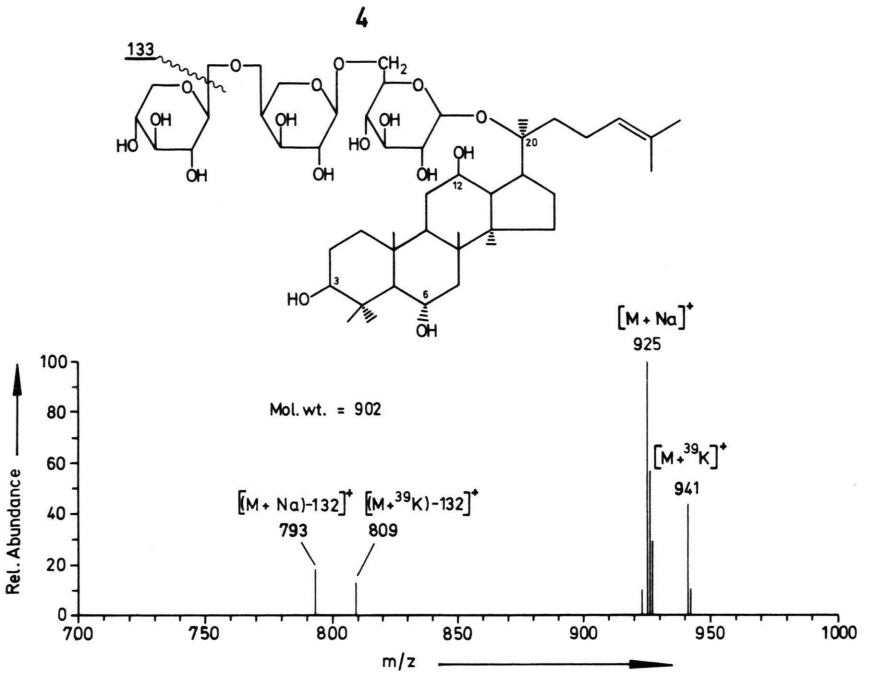


Fig. 5. FD mass spectrum of **4** obtained at approximately 28 mA emitter heating current as described for **1**. Only a short desorption time was observed (< 20 s) which allowed only the registration of three spectra by the data system.

agreement with this fact. Similarly to the FD processes indicated in the mass spectrum of **2** in Fig. 3 in solution partial acidic hydrolysis of **2** gave diosgenin-3-O-β-D-glucopyranoside (trillin) and diosgenin 3-O-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranoside [16]. It was of interest to compare the competitive cleavages of terminal xylose and glucose as explained above for **1** with the loss of these sugars from the same tetrasaccharide linked to a different aglycone. In Fig. 4 the FD mass spectrum of gitogenin 3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (= gitogenin 3-O-β-lycotetraoside = F-gitonin), **3**, is displayed. The characteristic FD signals can be described as follows:

1. The FD spectrum of **3** shows the $[M + Na]^+$ ion at *m/z* 1073 as the base peak. The $[M + {}^{39}K]^+$ ion at *m/z* 1089 is also found but with low relative abundance.

2. As observed for **1** and **2** no cleavage of the galactosidic bond at C-3 of the aglycone occurs.

3. The loss of terminal xylose is indicated by the intense ion at *m/z* 941 (14% relative abundance). In contrast, the cleavage of the terminal glucopyranosidic bond yields only a weak ion at *m/z* 911 (5% relative abundance). Furthermore the loss of both terminal sugars, xylose and glucose from the $[M + Na]^+$ ion gives a very weak signal at *m/z* 779. As may be inferred from Fig. 1, the sugar elimination observed for compound **3** is qualitatively quite similar to that of tomatine and the loss of the pentose xylose prevails as for **1** and **2**.

4. In the case of the water elimination from the $[M + Na]^+$ ion leading to the fragment at *m/z* 1057 (11% relative abundance), the hydroxyl group at C-2 of the aglycone should be considered.

In compound **4**, 20-O-[β-xylopyranosyl-(1 → 4)-α-arabinopyranosyl-(1 → 6)-β-glucopyranosyl]-20 S-protopanaxatriol (= chikusetsusaponin L5) [27], a trisaccharide (xylosyl-arabinosyl-glucose) is bound to a tertiary hydroxyl group in position C-20 of the aglycone. From the processes described above it can be deduced that in FD-MS for this saponin it is very likely that the bond cleavage of the glycoside takes place preferentially at the terminal xylose rest. This corresponds with the observation in the FD mass spectrum of **4** (Fig. 5) which shows only the loss of 132 mass units from the $[M + Na]^+$ ion and, considerably less abundant, from the $[M + {}^{39}K]^+$ ion.

This mass spectrometric behaviour of C-20 hydroxyl saponins is entirely different under electron impact conditions when the corresponding acetate- or TMS-derivatives were investigated. For instance, the EI mass spectrum of 6,20-di-O-β-D-glucosyl-20 S-protopanaxatriol (= ginsenoside-Rg_1), **5*** and related triterpene-saponin-acetates [8] as well as the trimethylsilyl-ether-derivatives of the dammarane type [10] have been studied. These compounds gave abundant, characteristic ions for the loss of the sugar molecules from the hydroxyl group in position 20 as follows for example: double bond type fragment $[M-(330 + H_2O)]^{+\cdot}$ and cationtype fragment $[M-(330 + OH)]^+$ in the mass spectrum of ginsenoside-Rg_1 deca-acetate [8] (see Scheme 2).

The mass spectrometric information obtained from the loss of the xylosyl-arabinosyl-glucose unit was not observed. Also, partial acidic hydrolysis in solution gave a fast cleavage at C-20 and pronounced sugar loss from the aglycone part in dammarane saponins.

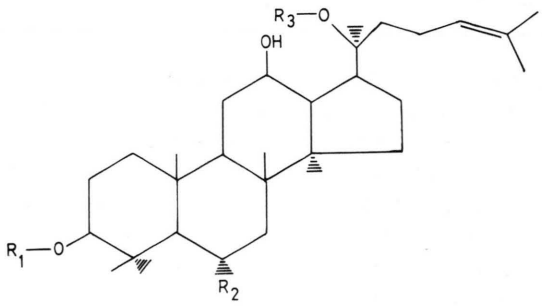
Similarly, compound **6**, 6-O-α-rhamnopyranosyl (1 → 2)-β-glucopyranosyl 20 S-protopanaxatriol 20-O-β-glucopyranoside (= ginsenoside R_e) [27] is easily hydrolyzed. Here the sugar moieties are bound to the aglycone at the C-6 and C-20 position.

Again preponderantly the glucopyranosyl group at C-20 is cleaved off in solution. In contrast, the FD mass spectrum of **6** showed only a highly abundant $[M + Na]^+$ ion at m/z 969 and elimination of the sugar from the tertiary hydroxyl group is not observed. Obviously this behaviour represents another characteristic feature of FD-MS of glycosides. The procedure of pumping thermal energy into the molecule in order to induce fragmentation is limited to these smaller molecules by their volatility. If the sample molecules desorb too rapidly from the emitter surface as observed for **4** and **6**, little or no sequence-specific fragments can be produced.

Another difficulty in sequencing oligoglycosides arises if the sample contains almost no alkali salts [43]. When model substances of very high purity are analyzed by FD-MS *i. e.* even small traces of alkali salts are removed, the spectra are characterized by the $[M + H]^+$ ion and its fragments. For instance compound **7**, 20 S-protopanaxadiol 20-O-β-D-glucopyranoside (= comp. K) [30] gave at emitter heating currents *above* BAT (17 mA) the $[M + H]^+$ ion at m/z 623 with only 23% relative abundance. Now, owing to the considerable thermal stress the cleavage at C-20 was observd and the loss of the glucopyranosyl rest was indicated by the signal at m/z 460 with 5% relative abundance. However, much more pronounced were the ions due to consecutive, thermal water eliminations at m/z 442 (42% rel. abundance) and m/z 424 (which is the base peak). In the FD spectrum of 20 S-protopanaxatriol 20-O-β-D-glucopyranoside analogous types of ion occurred. Thus, it is demonstrated that in these spectra optimal FD conditions for molecular weight *and* sequence determination were not fulfilled. Since the attachment of protons to the molecule generates less stable quasi-molecular ions than cationization by alkali cations, for samples of very high purity, it is advisable to add minute amounts of lithium or sodium salts [31 to

* The structures of compounds **5, 6,** and **7** are given in Scheme 1.

Scheme 1.

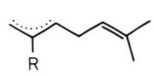


5 : R_1 = H
R_2 = O-ß-glucopyranose
R_3 = ß-glucopyranose

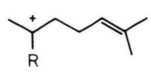
6 : R_1 = H
R_2 = O-ß-glucopyranose(2→1)α-rhamnopyranose
R_3 = ß-glucopyranose

7 : R_1 = H
R_2 = H
R_3 = ß-glucopyranoside

Scheme 2.



Double bond type fragment



Cationtype fragment

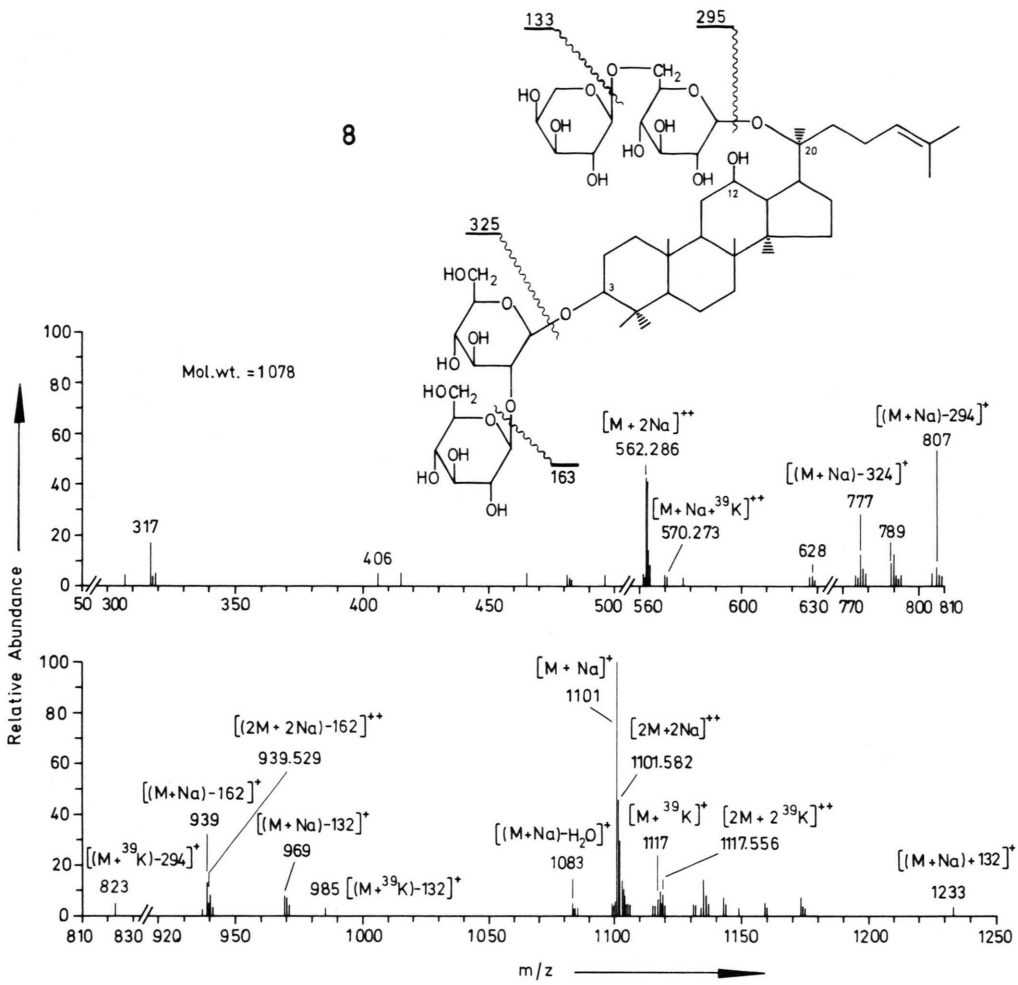


Fig. 6. FD mass spectrum of **8**. Electrical recording by two different instruments and operators (see Methods A *and* Methods B) gave qualitatively similar results, *i. e.* quasimolecular and fragment ions at the same nominal masses but with different relative abundances.

33]. It should be mentioned, however, that sugar-containing samples from biological material such as natural products in our experience almost always contained alkali salts in concentrations which were sufficient (or too high!) to perform cationization.

On careful selection of appropriate parameters for FD-MS, such as ionization efficiency of the emitter, its mechanical and electrical adjustment, the sample temperature and alkali content, and a sensitive, preferably integrating detection system, highly informative data were obtained from the spectra. The potential of the technique for molecular weight determination *and* sequence information is shown in Fig. 6. FD spectra of compound **8**, 3-O-β-glucopyranosyl (1 → 2)-β-glucopyranosyl-20 S-protopanaxadiol 20-O-β-arabinopyranosyl (1 → 6)-β-glucopyranoside (= ginsenoside Rb_2), [34] were recorded electrically between 27–30 mA emitter heating current and averaged by the data system. The characteristic FD signals of **8** can be described as follows:

1. The $[M + Na]^+$ ion at m/z 1101 is the base peak; in addition the $[M + {}^{39}K]^+$ ion is found with 10% relative abundance.

2. Loss of water from the sodium cluster plays only a minor role and gives the $[(M + Na) - H_2O]^+$ ion at m/z 1183 with 5% relative abundance only.

3. Intense doubly charged ions are observed at *m/z* 562.286 [35] for the $[M + 2Na]^{2+}$ ion and at *m/z* 570.273 for the $[M + Na + {}^{39}K]^{2+}$ ion. In addition, at a slightly different ratio of accelerating voltage/electric sector voltage, *i. e.* at higher mass resolution, a novel type of FD ions, namely $[2M + 2Na]^{2+}$ and $[2M + 2K]^{2+}$ were detected which are superimposed to the singly charged quasimolecular ions. Thus, seven different ion series can be used for unambiguous assignment of the *molecular weight.*

4. As may be inferred from the structure of **8** in Fig. 6 the sequence-specific ions allow to establish the complete *sequence* of the sugar units in the dammarane-type saponin. Loss of terminal arabinose indicate the $[(M + Na) - 132]^+$ and $[(M + {}^{39}K) - 132]^+$ ions at *m/z* 969 and *m/z* 985 respectively. Of comparable relative abundance is the $[(M + Na) - 162]^+$ ion at *m/z* 939 which corresponds to the cleavage of terminal glucose. In the same mass range but at higher mass resolution, a doubly charged ion is again detected at *m/z* 939.529 which is explained as $[(2M + 2Na) - 162]^{2+}$ ion. The ratio for the elimination of arabinose and glucose is approximately 1 : 1.5 after averaging the FD spectra at the end of the complete desorption of the sample.

The glucosyl-glucose bound to the aglycone at position C-3 is split off and gives the $[(M + Na) - 324]^+$ ion at *m/z* 777 with 12% relative abundance. Interestingly, a further loss of water leading to *m/z* 759 is not observed. The corresponding ion is clearly detected, however, when the other diglycoside which is linked at C-20 to the aglycone is eliminated. Firstly, the $[(M + Na) - 294]^+$ ion at *m/z* 807 is a weak fragment ion of only 7% relative abundance and secondly, loss of water leading to *m/z* 789 is quite intense. Thus the preferable water elimination from C-20 as already suggested for compound **7** is supported.

5. It is noteworthy that in the mass range above the quasimolecular ions, a number of FD signals indicate minor impurities in the present sample of **8**. In particular the $[(M + Na) + 132]^+$ ion might be a hint for an additional pentose bound to the saponin. However, it is possible that this ion originates from a cleavage from the dimeric $[2M + Na]^+$ ion. The other possibility, namely cationization of the molecule by an ion of 132 mass units can be excluded since this ion is not observed in the FD spectrum of **8**.

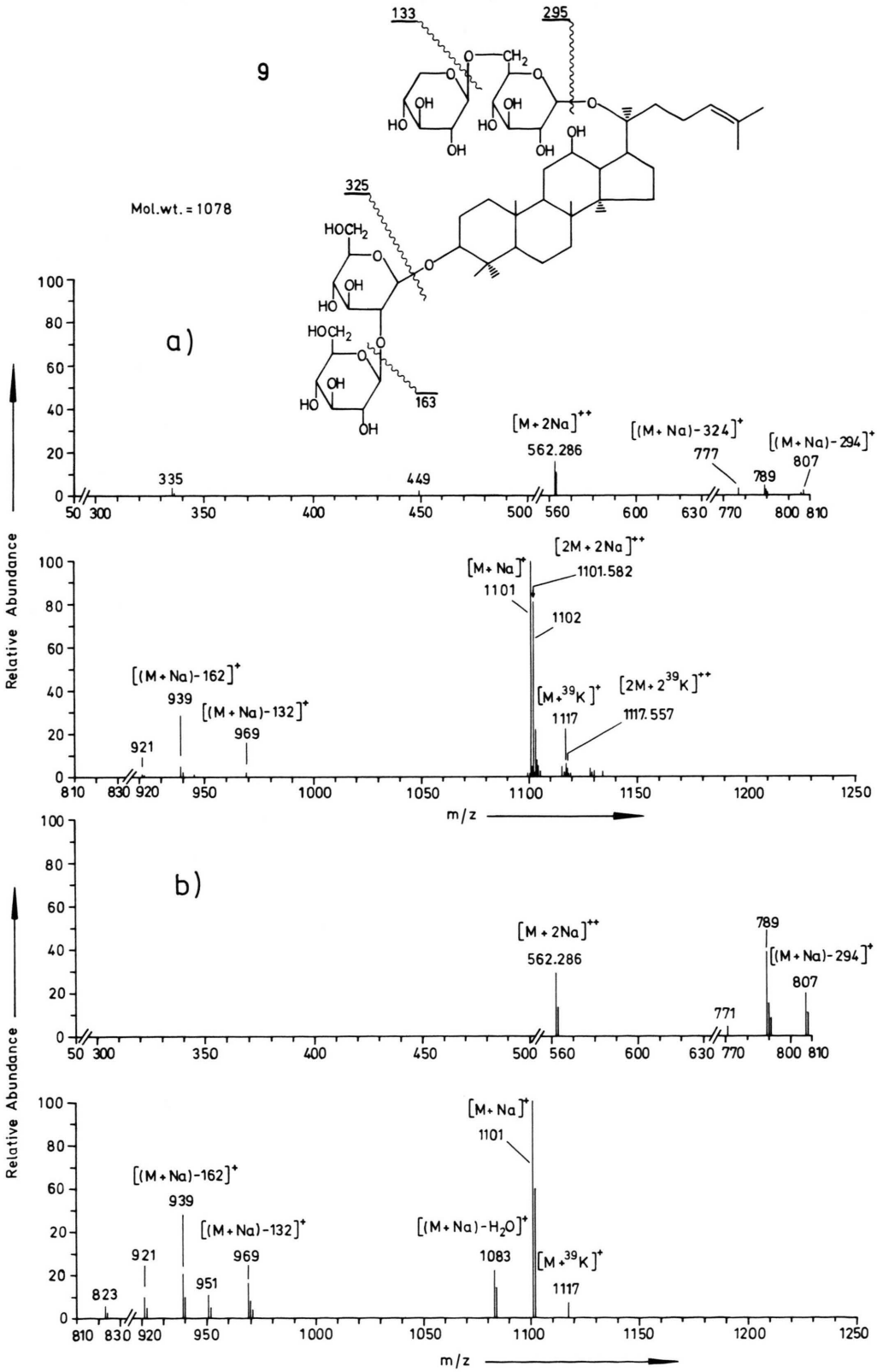
Reproducibility of FD spectra of natural products

When the FD spectrum of **8** was recorded under the experimental conditions described for the JEOL instrument (see Methods, A), qualitatively similar information could be obtained as from Fig. 6 (see Methods, B). This result is very promising in view of the fact that the group in Fukuoka started with FD-MS just a few months ago, one of the first commercial instruments was used and emission-controlled FD [36, 37] could not be performed since it is commercially not yet available. Although the repeatability of FD spectra (same sample, operator, instrument and experimental conditions) has been explored, little is known about the reproducibility in FD-MS, that is, using the same sample and similar experimental conditions but different instruments run by different operators.

Fig. 7 allows a first comparison of the FD spectra of a natural product, **9**, which were obtained in Bonn (Fig. 7a) and Fukuoka (Fig. 7b) meeting the requirements of reproducibility. There is one important difference in these spectra of 3-O-β-D-glucopyranosyl (1 → 2)-β-D-glucopyranosyl-20 S-protopanaxadiol 20-O-β-D-xylopyranosyl (1 → 6)-β-D-glucopyranoside (= ginsenoside Rb_3), **9**. Owing to a higher sample temperature (> BAT) the fragmentation in the FD spectrum of **9** in Fig. 7b is more pronounced. For instance, water elimination occurs from the quasimolecular ion resulting in a signal at *m/z* 1083 with about 22% relative abundance in Fig. 7b but is not observed at all in Fig. 7a. Accordingly, the sequence-specific fragment ions are in general also more intense. As noted above for the isomeric compound **8**, loss of terminal xylose (*m/z* 969) and terminal glucose (*m/z* 939) is clearly detected in both spectra and the ratios for the terminal cleavages are in good agreement. The signals for the loss of both disaccharidic chains at C-3 and C-20 are present in Fig. 7a but one signal at *m/z* 777 for the $[(M + Na) - 324]^+$ ion is missing in Fig. 7b. However, both spectra are in essentially good agreement. The high relative abundances of the singly and doubly charged quasimolecular ions are, in our opinion, an especially good demonstration of the reproducibility in FD-MS.

Conclusion

Beyond molecular weight determination, FD-MS has been shown to be a useful tool for the elu-


9
Mol.wt. = 1078
133
295
325
163
a)
b)
Relative Abundance
m/z
[M+2Na]++
562.286
[(M+Na)-324]+
777
[(M+Na)-294]+
807
789
335
449
[2M+2Na]++
[M+Na]+
1101
1101.582
1102
[(M+Na)-162]+
939
[(M+Na)-132]+
969
921
[M+39K]+
1117
[2M+239K]++
1117.557
[(M+Na)-H2O]+
1083
951
823

cidation of sugar sequences in steroid- and dammarane-saponins. The analogy of high field solvolysis to the processes in solution chemistry is useful for qualitative interpretation and for the better understanding of surface/field-induced mechanisms. Quantitative data *e. g.* hydrolysis rates from acidic hydrolysis in solution cannot be transferred.

For oligoglycosides, loss of terminal sugar prevails as a general rule. The cleavage of the O-glycosidic bond between the aglycone-linked sugar and the aglycone is of small intensity or not observed owing to stabilization of the ether-bridge by the aglycone. This dominant elimination of the terminal sugar unit was likewise observed for other oligoglycosides such as rutin, naringin, hesperidin [38] and sarsapogenin-O-β-D-xylopyranosyl (1 → 2)-β-D-galactopyranoside [39]. In cases where two oligoglycoside chains which differ by their terminal sugar are bound to the aglycone, loss of these sugars appears to be governed by the activation energies for the corresponding bond cleavages. This is supported, for instance, by the dependence of the ratios of sequence-specific ions on the applied thermal energy (heating current). Some trends such as the preferable loss of terminal xylose or the differentiation of α- and β-glucopyranosides in FD [40] emerge but have to be documented for a larger number of examples before generalizations and rules for FD-MS of oligoglycosides can be put forward. An updated and comprehensive survey of the analytical applications of FD-MS in biochemistry, medicine, and environmental research has recently been given [41] and the advantages and pitfalls of the technique for investigations of O- and N-glycosides were outlined.

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Fig. 7. a) FD mass spectrum of **9** recorded with the Varian MAT 731 instrument in Bonn (see Methods B). Eight spectra were averaged in an emitter heating current interval between 27 to 30 mA. b) FD mass spectrum of **9** recorded with the JEOL-D-300 instrument in Fukuoka. The spectrum represents the results of a single magnetic scan.

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