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FIELD DESORPTION MASS SPECTROMETRY OF NATURAL PRODUCTS—II†

PHYSIOLOGICALLY ACTIVE PENNOGENIN—AND HEDERAGENIN-GLYCOSIDES

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Abstract—Field desorption mass spectra of underivatized saponins, natural oligoglycosides of physiological activity, showed intense ions formed by attachment of alkali cations to the neutral molecule and to structurally highly significant subunits. This cationization was generated by small amounts of alkali salts which were present in samples after isolation from biological material. When the mass spectra were produced by laser-supported desorption and were recorded at high resolution on vapor-deposited AgBr plates unambiguous determination of the molecular weghts (in the mass range from 700 to 1400) was performed. Moreover, ions formed by direct bond cleavages in the oligosaccharide moiety of the natural saponins clearly gave information about the sequence of the sugar units in the molecule and their individual chemical structures. The formation of these fragments in FD–MS is discussed in relation to the well established mechanism of glycosidic bond cleavage by acidic solvolysis. In principle this comparison showed the usefulness of this approach for the interpretation of FD mass spectra of substances containing heteroatom linkages e.g., glycosides, esters, amides, imines, mercaptans, etc.

The structural elucidation of the pennogenin-glycoside which was isolated from fresh rhizomes of *Trillium kamtschaticum Pall* has previously been reported.[1] From this study it became clear that pennogenin is not always the artefact as described by Marker *et al.*[2] but is indeed found as an oligoglycoside (steroid saponin) in plants. Later seventeen oligoglycosides of the oleanane type were isolated from the fruits of *Akebia quinata Decne* and their structures (triterpene saponins) were established showing that the aglycone has a glycosidic bond in the 3- and/or the 28-position.[3]

The common properties of saponins are characterized by marked foam formation in aqueous solutions, by hemolytic activity, by strong toxicity towards fish, by complex formation with cholesterol and pronounced antifungal action.[4] Since the underground parts of the *Trillium* plant are used in the treatment of stomach diseases and the fruits of *Akebia quinata* showed diuretic effects in humans, some pharmacological action could be expected for the oligoglycosides described in this study. However, from the analytical point of view molecular weight determination and structure elucidation of these natural products have been hampered by their complicated structure and high polarity of the saponins. In addition considerable difficulties arise in the procedures for the isolation of these substances in the pure state.

In order to exploit the differences between the oligoglycosides, their peracetates and permethyl ethers were investigated systematically by electron impact (EI) mass spectrometry (MS) using high resolution and accurate mass measurements.[5,6] The mass spectra obtained showed no molecular ion $[M]^{+\cdot}$ for oligoglycosides larger than the tetraglycosides. In particular conventional mass spectrometry of the 28-*O*- and 3,28-*O*-glycosidal triterpenes of the oleanane type gave no information about the molecular weight.

In continuation of our recent report on field desorption (FD) MS of steroid- and triterpene saponins[7] we now demonstrate that FD–MS is a powerful tool not only in molecular weight determination but also in investigations of the structure of underivatized oligoglycosides.

RESULTS AND DISCUSSION

The FD mass spectrum of pennogenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**1**) in Fig. 1 recorded photographically and at high resolution showed an intense protonated molecule at *m/e* 739.427. The thermal/field induced loss of water from this $[M+H]^+$ ion is very pronounced and gives the base peak of the spectrum at *m/e* 721.416. It is assumed that the attachment of the proton leading to subsequent water elimination occurs at the OH function at C-17. This is supported by the following three observations.

(a) An intense ion is found at an accurate mass of 593.369 which is consistent with an $[(M+2H)-A]^+$ ion. This means that the cleavage of the terminal sugar unit is initiated by proton attack at the oxygen linking the sugar moieties as is commonly observed in FD–MS of oligoglycosides.[8] This process, to some extent analogous to the acidic solvolysis of glycosides in solution chemistry,[9] is depicted in Scheme 1. The $[H]^+$ ions may originate either from the solvent or may be generated by thermally induced reactions in the high field chemistry on the emitter surface.[10] Alternatively the proton donor can be added to the sample in aqueous solution in order to

†Part I: H.-R. Schulten, T. Komori and T. Kawasaki, *Tetrahedron* **33**, 2595 (1977).

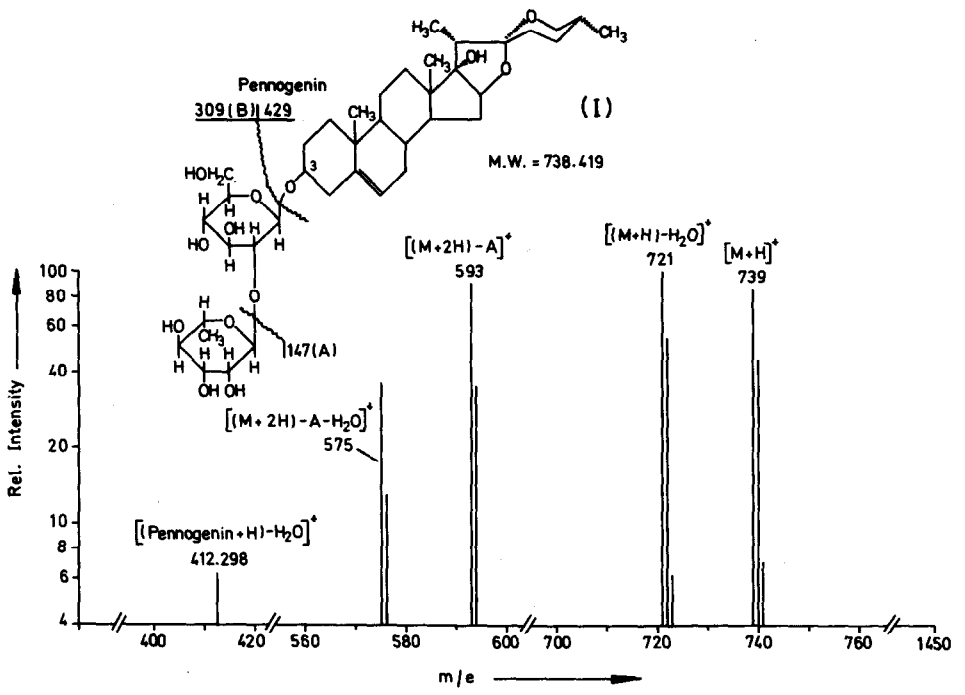


Fig. 1. FD mass spectrum of 1.

promote hydrolysis.[22] Attachment of the proton to the basic sites in the molecule occurs producing the $[M+H]^+$ ion. This ion is desorbed from the emitter and is characteristic for the FD spectra of sugars and other polar substances if the competing (and favorable) process of cationization by alkali cations[11,12] does not prevail. However, as depicted in Scheme 1 specific proton attack on the glycosidic oxygen and charge localization (structure a) can induce a characteristic electron shift from the ring oxygen resulting in the formation of the glycosyl ion (b). This $[(M+H)-ROH]^+$ ion is almost always found in the FD-MS of sugars and sugar derivatives[13] if desorption is performed close to or above the best anode temperature (b.a.t.).[14] Since protonation-induced cleavage of the glycosidic bond occurs not only at the terminal sugar but also at other linking heteroatoms of the molecule, information about the sequence of the sugar units in the oligoglycoside molecule can be obtained (see below).

(b) The fragment at *m/e* 575.358 is assigned to the $[(M+2H)-A-H_2O]^+$ ion. From the occurrence of this ion and a weak signal at *m/e* 147.066 for **A** it becomes clear that for compound **1** the loss of water does not originate from the terminal rhamnose moiety.

(c) The assumption that water elimination takes place preponderantly from the OH group at C-17 is supported by the occurrence of a weak but highly significant ion at *m/e* 412.298 which indicates both the loss of two sugar molecules and loss of water from the protonated aglycone pennogenin.

It is noteworthy that the ions at *m/e* 593 and *m/e* 575 corresponding to the protonation-induced bond cleavage at the rhamnose moiety (**A**) are shown with a high relative abundance in the spectrum. In contrast an ion arising by analogous cleavage between glucose and the aglycone is formed only with minor intensity and after loss of water from the aglycone. Similar observations of the preferential rupture of the rhamnose/glucose bond have been made in the FD spectra of steroid and triterpene saponins.[7] Because it is well known that in solution chemistry α-rhamnosides are hydrolysed 5–10 times faster than β-glucosides,[15] two conclusions important for the analytical application of FD-MS emerge. Thus these findings support the validity of the proposed mechanism of protonation-induced cleavage of the glycosidic bond in FD-MS and its similarity to solution chemistry. Additionally information about the nature of the sugar units in the oligoglycoside can be derived from the ions at *m/e* 147 (**A**) and *m/e* 309 (**B**) in the FD mass spectrum of **1**. A signal for the aglycone, pennogenin (mass 430), is not found.

Owing to the negligible contamination of the natural product by alkali salts and the desorption program employed, no products of cationization of compound **1** by alkali ions were detected. In contrast the FD mass spectrum of hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**2**, Fig. 2) shows exclusively ions which contain Na. The attachment of Na cations to the intact molecule produced the base peak of the spectrum.

Accurate mass measurements (*m/e* 773.445) give a first hint for the assignment of the molecular weight. Since $[M]^+$ or $[M+H]^+$ ions or any fragment ions formed, for example, by the frequently-observed loss of water or formic acid from protonated carboxylic acids[8] are absent, the extraordinary high stability of charged particles generated by alkali attachment is again demonstrated. In consequence, however, structural information is not available in this case as the characteristic losses of sugar moities are not observed. A unique type of ion occurs at *m/e* 409.208. Na-salt impurities present in trace amounts exchange the proton of the carboxylic acid at C-28 to form the organic Na-salt. Obviously this process takes place on the surface of the emitter when the emitter temperature rises and the solvent (MeOH) is evaporated. The saponin Na-salt then generates a doubly-charged

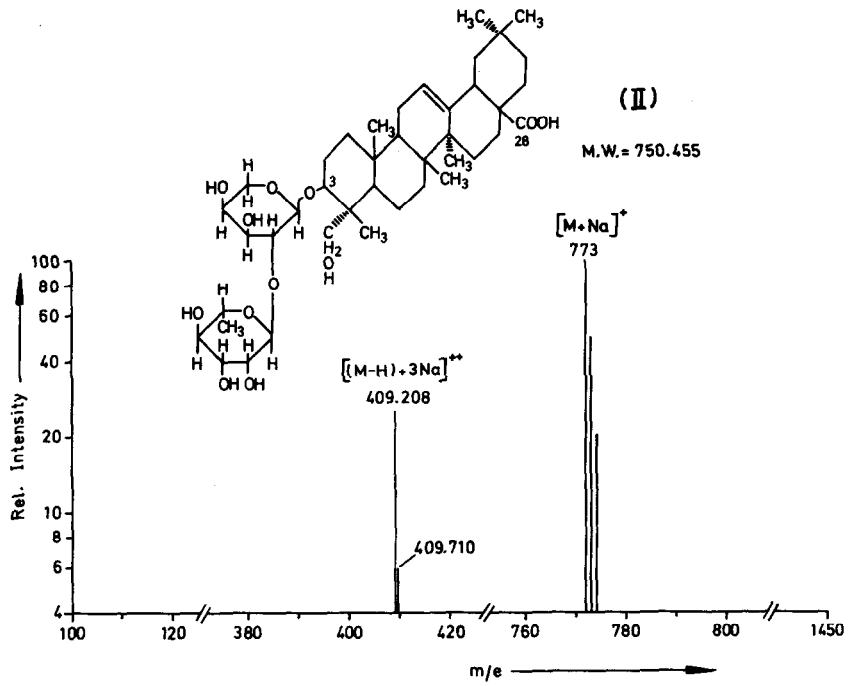


Fig. 2. FD mass spectrum of 2.

cluster ion whose accurate mass confirms the presence of a carboxylic function and the suggested molecular weight. The usefulness of multiply charged ions for the determination of the molecular weight has been described previously.[16,17] That this point is particularly relevant for the FD analysis of heteroatom-linked natural products such as peptides, nucleotides and oligosaccharides is demonstrated for an oligoglycoside in Fig. 3. For compound (3), pennogenin 3 - *O* - α - L - rhamnopyranosyl - (1→4) - α - L - rhamnopyranosyl - (1→4) - [α - L - rhamnopyranosyl - (1→2)] - β - D - glucopyranoside again the base peak of the FD spectrum is generated by the $[M+Na]^+$ ion at *m/e* 1053.525. Compound 3 has *four* different possibilities for a fission of the glycosidic bonds by the protonation-induced cleavage described in Scheme 1.

1. Loss of the terminal rhamnose units A_1 or A_2 and cationization by Na-salt impurities leads to the [(M+

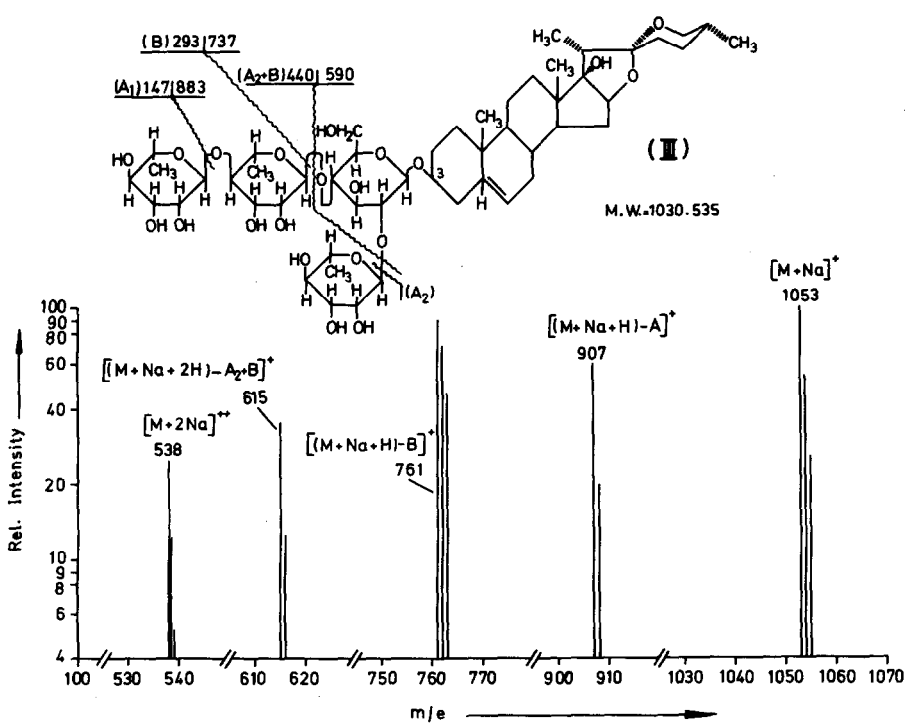
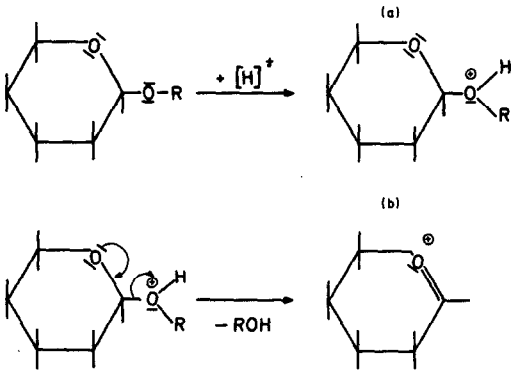


Fig. 3. FD mass spectrum of 3.



Scheme 1. Proposed mechanism for the protonation induced cleavage of the glycosidic bond in the field desorption process. The most intense ions are formed by alkali cation attachment to the intact molecule M and to the sequence-specific ROH species. Elimination of the terminal sugar moieties (A, B, C, etc.) is indicated by the production of the ion (b) and gives complementary structural information (8).

$Na+H)-A]^+$ ion at *m/e* 907.467. (In general the sugar moieties are abbreviated to **A** (monosaccharide), **B** (disaccharide) and **C** (trisaccharide). Whether the loss of A_1 or of A_2 is the dominating process cannot be decided from the FD spectrum as both processes give isomeric ions.

2. Cleavage of the rhamnopyranosyl-(1→4)-rhamnopyranosyl part (**B**) from compound **3** gives the most intense fragment ion $[(M+Na+H)-B]^+$ at *m/e* 761. A less probable contributor to *m/e* 761 is an ion of the same accurate mass formed by the losses of the two terminal rhamnose units A_1 *and* A_2 generated by double proton attack at the two different glycosidic oxygens and cationization of the residual part of the molecule.

3. The occurrence of double cleavages is not so pronounced (also in resemblance to solution chemistry) and is illustrated by the lower relative abundance of the ion at 615.351, formed by the loss of fragments **B** *and* A_2 from **3**. However, this ion affords evidence for the sequence of the sugars in the oligoglycoside.

4. As with compound **1** the cleavage at the rhamnose moieties is again the dominating process. Ions for the fourth possible fission, namely between the glucosyl oxygen and the aglycone are not observed. Admittedly a stabilising effect of the aglycone on the glycosidic bond at C-3 has not been taken into consideration and presently no oligoglycosides with glycosidic bonds of rhamnose in this position and terminal glucose units are available for assessment of this effect.

The intense doubly charged $[M+2Na]^{2+}$ ion at *m/e* 538.257 again supports the correct assignment of the molecular ion. Often the multiply charged clusters of the molecule are found not only with Na, but also with K or with both alkali cations. For example compound **4**, norarjunolic acid 28 - O - α - L - rhamnopyranosyl - gentiobioside, gave no $[M]^+$ or $[M+H]^+$ ions. The $[M+Na]^+$, $[M+{}^{39}K]^+$ and $[M+{}^{41}K]^+$ ions were recorded with saturated blackening of the photoplate and their corresponding doubly charged ions unambiguously confirm the molecular weight of the substance investigated. The relative intensities and accurate masses of these ions are listed in Table 1. Elimination of water is a minor process. From the structure of compound **4** (Fig. 4) it is clear that observance of three protonation induced cleavages, as explained in Scheme 1, would determine the sequence of the sugars in the oligoglycoside chain. Indeed all these ions are found (Table 1) cationized by

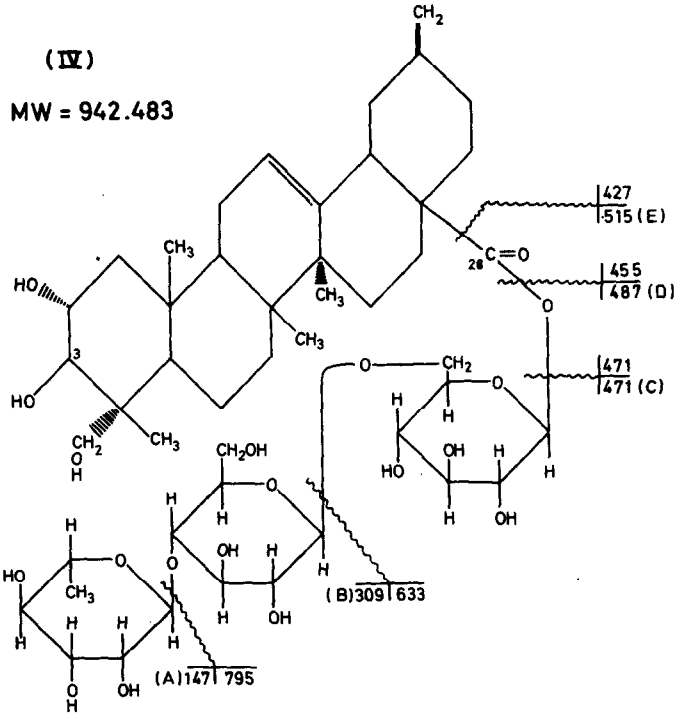


Fig. 4. Structure of compound **4** showing characteristic cleavages in the molecule and the corresponding nominal masses. The theoretical accurate masses and the relative intensities of the ions recorded are given in Table 1.

Table 1. Significant ions in the high resolution FD mass spectrum of compound IV for the determination of the molecular weight and the structure

Type of Ion	Relative Intensity*	Accurate Mass
$[M+^{41}K]^+$	+++	983.444
$[M+^{39}K]^+$	+++++	981.446
$[M+Na]^+$	+++++	965.472
$[M]^+$	-	942.482
$[M+H]^+$	-	943.490
$[M+2Na]^{++}$	+++++	494.231
$[M+Na+^{39}K]^{++}$	++++	502.218
$[M+2^{39}K]^{++}$	+	510.205
$[M+2^{41}K]^{++}$	-	512.203
$[(M+Na)-H_2O]^+$	++	947.462
$[(M+^{39}K)-H_2O]^+$	+	963.436
$[(M+^{41}K)-H_2O]^+$	+	965.434
$[(M+Na+H)-A]^+$	+++++	819.414
$[(M+^{39}K+H)-A]^+$	++++	835.388
$[(M+^{41}K+H)-A]^+$	+	837.386
$[(M+Na+H)-B]^+$	+++	657.361
$[(M+^{39}K+H)-B]^+$	++	673.335
$[(M+^{41}K+H)-B]^+$	-	675.334
$[(M+Na+H)-C]^+$	+++	495.309
$[(M+^{39}K+H)-C]^+$	+++	511.283
$[(M+^{41}K+H)-C]^+$	+	513.281
$[(M+Na+H)-D]^+$	+++++	479.314
$[(M+^{39}K+H)-D]^+$	+++	495.288
$[(M+^{41}K+H)-D]^+$	++	497.286
$[(M+Na+H)-E]^+$	++++	451.319
$[(M+^{39}K+H)-E]^+$	+++	467.293
$[(M+^{41}K+H)-E]^+$	+	469.291

* The relative intensities on the photoplate are given in five degrees:

0 - 20 % rel. int.	=	+
20 - 40 % rel. int.	=	++
40 - 60 % rel. int.	=	+++
60 - 80 % rel. int.	=	++++
80 -100 % rel. int.	=	+++++ .

Na and with one exception (*m/e* 675.334) by both K isotopes. Further the preferential fission of the rhamnose moiety (A) is reflected in the considerably higher intensity of the ion $[(M+Na+H)-A]^+$ (rel. int. 81%) as compared with the ion $[(M+Na+H)-B]^+$ (rel. int. 66%). As might be expected by analogy with the solution chemistry of acylglycosides splitting at the glycosidic oxygen between norajunolic acid and the oligosaccharide chain by acidic solvolysis is found to be a prominent and competing mechanism leading to the $[(M+Na+H)-C]^+$ ion (Table 1) and an intense $[(M+2Na+H)-C]^{2+}$ ion at *m/e* 259.149 which is the doubly charged cluster ion of norarjunolic acid Na-salt.

The complete FD mass spectrum of compound **5**, 3-O-β-D-glucopyranosyl-(1→2) - [α-L-rhamnopyranosyl - (1→4)]-α-L-arabinopyranosyl - hederagenin 28-O-α-L-rhamnopyranosyl - (1→4) - β - D - glucopyranosyl-(1→6)β-D-glucopyranoside has been reported previously.[7] As can be inferred from the structure of **5** (Fig. 5) and the high resolution data presented in Table 2 all sequence-specific fragments are found. Protonation-induced cleavage at the glycosidic oxygens indicates the loss of one to six sugar units from the oligoglycoside. In general the relative intensity of the ions produced decreases with increasing number of bond cleavages which once more is in analogy to solution chemistry. Fission of the rhamnose bond is preferred as described for the other saponins. The molecular weight is determined by singly and doubly charged ions and the chemical nature of the aglycone hederagenin is marked by the ion due to the loss of

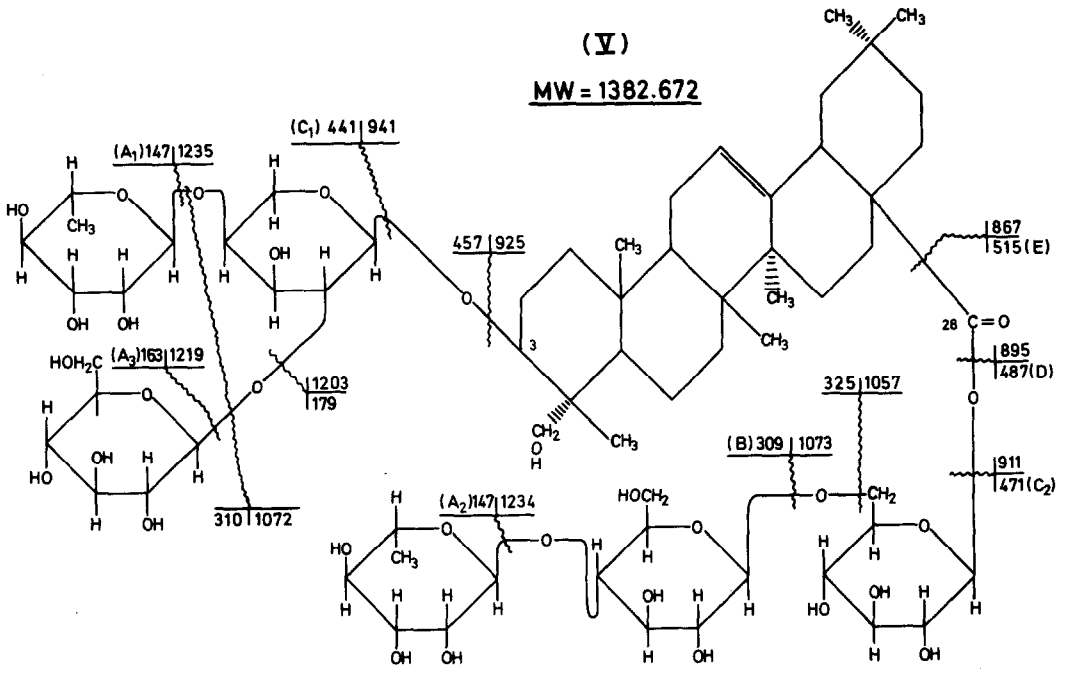


Fig. 5. Structure of compound **5**. The sequence-specific bond cleavages are indicated by their abbreviations and nominal masses. The theoretical accurate masses and the relative intensities of the ions found are given in Table 2.

Table 2. Significant ions in the high resolution FD mass spectrum of compound V for the determination of the molecular weight and the structure

Type of Ion	Relative Intensity*	Accurate Mass
$[M+Na]^+$	++	1405.662
$[M]^+$	-	1382.672
$[M+H]^+$	-	1383.680
$[(M+Na+H)-A_1]^+$	+++	1259.604
$[(M+Na+H)-A_2]^+$		
$[(M+Na+H)-A_3]^+$	++	1243.609
$[(M+Na+2H)-A_1-A_2]^+$	+++	1113.546
$[(M+Na+2H)-A_1-A_3]^+$	+++	1097.551
$[(M+Na+2H)-A_2-A_3]^+$		
$[(M+Na+H)-B]^+$		
$[(M+Na+H)-C_1]^+$	+	965.509
$[(M+Na+2H)-A_1-B]^+$	+++	951.493
$[(M+Na+2H)-A_3-B]^+$	+++++	935.498
$[(M+Na+H)-C_2]^+$		
$[(M+Na+H)-D]^+$	++	919.485
$[(M+Na+H)-E]^+$	+++	891.490
$[(M+Na+2H)-A_1-C_2]^+$	++++	789.440
$[(M+Na+2H)-A_3-C_2]^+$	++++	773.445
$[(M+Na+2H)-A_1-D]^+$		
$[(M+Na+2H)-A_1-E]^+$	+	745.450
$[(M+Na+2H)-A_2-E]^+$		
$[(M+Na+2H)-C_1-C_2]^+$	+++++	495.345
$[(M+Na+2H)-C_1-D]^+$	++++	479.350
$[(M+Na+2H)-C_1-E]^+$	+	451.355

Table 2 (*Contd.*)

Type of Ion	Relative Intensity*	Accurate Mass
$[M+2Na]^{++}$	++	714.326
$[(M+2Na+H)-A_1]^{++}$ } $[(M+2Na+H)-A_2]^{++}$ }	++	641.297
$[(M+2Na+H)-A_3]^{++}$	+	633.299
$[(M+2Na+H)-B]^{++}$	+	560.270
$[(M+2Na+H)-C_1]^{++}$	++	494.249
$[(M+2Na+H)-C_2]^{++}$	++++	479.244

* Listed as in Table 1.

both triglycosides C_1 and C_2 at *m/e* 495.345. For the sake of simplicity the ions generated by attachment of K are not listed and only a few selected doubly charged ions are shown in Table 2.

Further sequence information is obtained from cleavages in the molecule eliminating the terminal sugars including the glycosidic oxygen (1203, 1057 and 915 in Fig. 5). Again the corresponding ions are formed by solvolysis and Na attachment and appear singly/doubly charged in the FD spectrum of V.

EXPERIMENTAL

The FD spectra were produced on a commercial Varian MAT 731 instrument (combined EI/FD ion source) by the photographic detection system wich vacuum evaporated AgBr photoplates (Ionomet, Waban, Mass., USA). The resolution obtained was about 15000 (at half peak width), and the average accuracy in the mass determination was ±10 millimass units. 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 μm dia. tungsten wires.[18] The distribution and morphology of the microneedles produced were as shown previously.[19] 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/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 an 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.[19] Field desorption was performed using direct heating of the FD emitter wire (approx. 15 mA) and simultaneous indirect heating[20] by a regulated Argon Laser (Mod. 166, Spectra Phys.). This technique improved the sensitivity of the mass spectrometer, particularly in the high resolution mode, and enabled a very fine adjustment of the desorption parameters.[21]

CONCLUSION

FD-MS enables not only the *molecular weight determination* of underivatized oligo-glycosides but yields important information about the sequence of the sugar moieties in the molecule. Thus the method shows useful results for *structural analysis* of natural products. The proposed mechanism of protonation induced cleavage of the glycosidic bond is in agreement with the produced ions and the well established process of acidic solvolysis in solution chemistry. The observed discrimination in the fissions of the rhamnosyl- and glucosyl-bond gives a first indication that statements about the configuration of the different sugar moieties in the oligoglycoside chain are possible.

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REFERENCES

[1] T. Nohara, K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull. Tokyo* **23**, 872 (1975).
[2] R. E. Marker and J. Lopez, *J. Am. Chem. Soc.* **69**, 2389 (1947).
[3] R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.* Tokyo **20**, 2143 (1972); *Ibid.* **24**, 1021, 1314 (1976).
[4] R. Tschesche and G. Wulff, *Fortschritte der Chemie organischer Naturstoffe* (Edited by L. Zechmeister, W. Griesbach and G. W. Kirby), p. 462. Springer, Wien, New York (1973).
[5] T. Komori, Y. Ida, Y. Mutou, K. Miyahara, T. Nohara and T. Kawasaki, *Biomed. Mass Spectrom.* **2**, 65 (1975).
[6] R. Higuchi, T. Komori and T. Kawasaki, *Chem. Pharm. Bull. Tokyo* **24**, 2610 (1976).
[7] H.-R. Schulten, T. Komori and T. Kawasaki, *Tetrahedron* **33**, 2595 (1977).
[8] H.-R. Schulten, *Methods of Biochemical Analysis* (Edited by D. Glick), Vol. 24, p. 313–448, and refs. cited. Wiley-Interscience, New York (1977).
[9] W. G. Overend, *The Carbohydrates* (Edited by W. Pigman and D. Horton) pp. 321, 324. Academic Press, New York (1972).
[10] H. D. Beckey, *Field Ionization Mass Spectrometry.* Pergamon Press, New York (1972); *Principles of Field Ionization and Field Desorption Mass Spectrometry.* Pergamon Press, New York (1977).
[11] F. W. Röllgen and H.-R. Schulten, *Org. Mass Spectrom.* **10**, 660 (1965).
[12] F. W. Röllgen and H.-R. Schulten, *Z. Naturforsch.* **30a**, 1685 (1975); H. J. Veith, *Angew. Chem.* Int. Ed. Engl., **15**, 695 (1976); J. C. Prome and G. Puzo, *Org. Mass Spectrom.* **12**, 23 (1977); F. W. Röllgen, U. Giessmann and H.-R. Schulten, *Adv. in Mass Spectrom. VII* (Edited by N. Daly). Heyden, London, in press (1977).
[13] H.-R. Schulten and D. E. Games, *Biomed. Mass Spectrom.* **1**, 120 (1974); H.-R. Schulten and H. M. Schiebel, *Z. Anal. Chem.* **280**, 139 (1976); *Nucl. Acids Res.* **3**, 2027 (1976).
[14] H. U. Winkler and H. D. Beckey, *Org. Mass Spectrom.* **6**, 655 (1972).

[15]W. G. Overend, C. W. Rees, J. S. Sequeira, *J. Chem. Soc.* 3429 (1962); J. N. Bemiller, *Advances in Carbohydrate Chemistry* (Edited by M. L. Wolfrom and R. S. Tipson), pp. 43, 52. Academic Press, New York (1967).
[16]H.-R. Schulten and H. D. Beckey, *Org. Mass Spectrom.* **9**, 1154 (1974).
[17]H.-R. Schulten and D. Kümmler, *Z. Anal. Chem.* **278**, 13 (1976).
[18]H.-R. Schulten and H. D. Beckey, *Org. Mass Spectrom.* **6**, 885 (1972).
[19]H. D. Beckey and H.-R. Schulten, *Angew. Chem.* Int. Edit. Engl., **14**, 403 (1975); and refs cited.
[20]H. U. Winkler and H. D. Beckey, *Org. Mass Spectrom.* **7**, 1007 (1973).
[21]H.-R. Schulten, W. D. Lehmann and D. Haaks, *Org. Mass Spectrom.* in press.
[22]F. W. Röllgen, H. J. Heinen and U. Giessmann, *Naturwiss.* **64**, 222 (1977).