Field Desorption and Fast Atom Bombardment Mass Spectrometry of Spirostanol and Furostanol Saponins from *Paris polyphylla* *

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The application of field desorption and fast atom bombardment mass spectrometry for molecular weight determination and structural elucidation of spirostanol and furostanol saponins isolated from Paris Polyphylla Sm. (Liliaceae) is reported. The capacity and limitations of these methods for sugar sequencing, differentiation of isomers and purity control of oligoglycosidic natural products are discussed.

Introduction

The structural elucidation of a number of Polyphyllins which were isolated from *Paris polyphylla* Sm. tubers have been reported [1-4].

The application of analytical techniques for the molecular weight determination and structure elucidation in the field of these free saponins has been hampered due to their molecular weights, complexity of structure and highly polar nature. The isolation of the saponins in pure state and sufficient quantity was thus very crucial in the studies of natural products. Investigations with electron impact (EI) mass spectrometry (MS) using high resolution and accurate mass measurements of the permethylated and peracetylated saponins have been reported [5]. The mass spectra of such derivates showed no peak for $[M]^{+\bullet}$, particularly for oligosaccharides with more than four sugar units, in the case of furostanol glycosides with even less than four sugars. To overcome these difficulties, the application of field desorption (FD) for saponins and some other glycosides has been studied [6-13] which gave information, not only regarding the molecular weight, but also for sequence determination of sugars. The utility of FD-MS in the elucidation of the structure of some new spirostanol and furostanol saponins is illustrated in the present investigation. In addition, the fast atom bombardment [14-16] (FAB) mass spectra of the saponins were recorded and their use as a complementary analytical tool evaluated. Thus the purpose of this paper is to demonstrate the potential of combined FD/FAB investigations of oligoglycosidic saponins.

Results and Discussion

Polyphyllin C

The FD mass spectrum of polyphyllin C (diosgenin-3-O-[α-L-rhamnopyranosyl $(1 \rightarrow 3)$]-β-D-glucopyranoside, **1**), displayed in Fig. 1 shows a signal at *m/z* 745 for the base peak and one at *m/z* 761 (low abundance) assigned to the $[M+Na]^+$ ion and $[M+{}^{39}K]^+$ ion respectively. It has been reported for FD-MS that in most cases of polar molecules, the mass peaks appear in association with either Na^+ or K^+ cations [6]. The high abundances of these molecular clusters are due to the fact that sodium and/or potassium salts are invariably introduced during the processing/isolation of the compound and this favours cationization. This process produces also a $[2M+Na]^+$ ion at *m/z* 1467 with 6% relative abundance. Thus three types of ion confirm the correct assignment of the molecular weight: $[M+Cat]^+$, $[M+2Cat]^{2+}$ and $[2M+Cat]^+$, where the cation (Cat) can be a proton or metal cation. Compound **1**

* Fied Desorption Mass Spectrometry of Natural Products Part XIV, for Part XIII see ref. [26].

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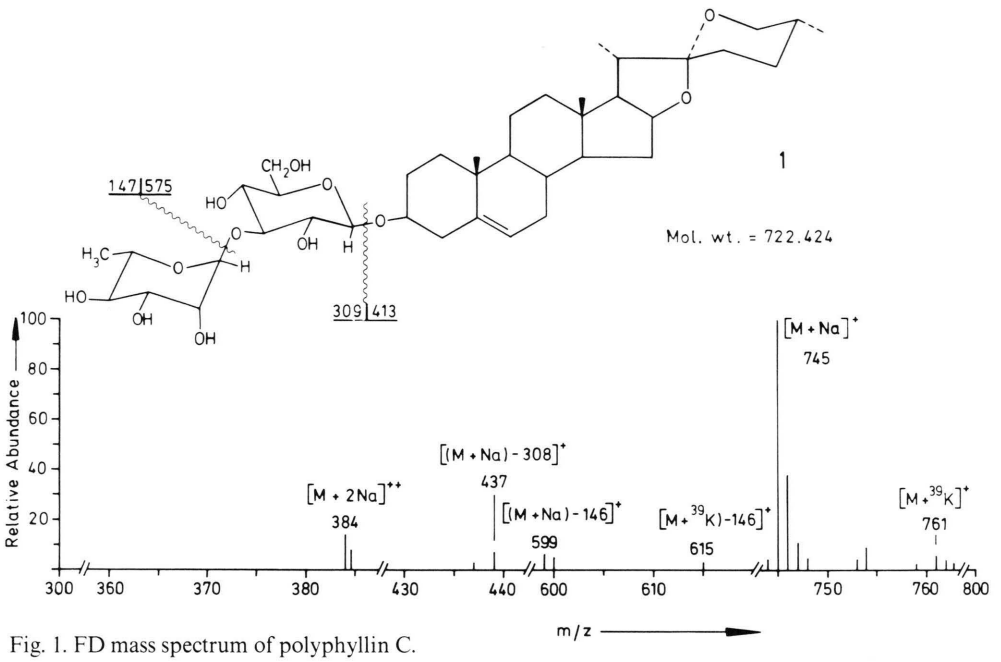

Fig. 1. FD mass spectrum of polyphyllin C.

shows a signal at m/z 599 due to the loss of 146 mass units from the $[M+Na]^+$ cluster, a result of cleavage of the terminal rhamnose unit from the molecule by a protonation mechanism analogous to acidic solvolysis [8]. In this proton induced cleavage of the glycosidic bond, specific proton attack on the glycosidic oxygen and charge localization can induce a characteristic electron shift from the ring oxygen resulting in the formation of a glycosyl ion. This type of ion is almost always found in the FD-MS of sugars and their derivates [9–13].

A weak intensity fragment ion at m/z 437 also is observed due to the loss of the rhamnosyl-glucoside unit from the $[M+Na]^+$ cluster and this reveals the steroid aglycone (mol. wt. 414). The low intensity is probably due to the strong β-linkage of the glucose to the diosgenin. As with acid hydrolysis, where D-glucose is hydrolysed 5–10 times slower than the L-rhamnose [17, 18], it was difficult to cleave the disaccharide from the point of this β-linkage in FD-MS. Furthermore, for steroid sapogenins, the stabilization of the glycosidic bond in 3-position of the aglycone is a generally observed phenomenon in FD-MS.

The FAB mass spectrum of **1** is shown in Fig. 2. In the recorded mass range, chemical noise from the glycerol matrix gives background ions with relative abundances between 0.5 and 6%. Nevertheless the most significance features of the FD results are clearly seen in this FAB spectrum. At m/z 723, the $[M+H]^+$ ion is registered with 46% relative abundance. Cationization by metal cations is not observed using the authentic specimen without any alkali salt as additive. The loss of the terminal rhamnose unit is indicated by a prominent $[(M+H)-146]^+$ ion at m/z 577. Water elimination from the steroidal aglycone (m/z 415) produces the base peak at m/z 397.

Polyphyllin D

The FD mass spectrum of polyphyllin D (diosgenin-3-O-α-L-rhamnosyl(1 → 3)[α-L-arabinofuranosyl(1 → 4)]-β-D-glucopyranoside, **2**) in Fig. 3 generates the base peak by the $[M+Na]^+$ ion at m/z 877. The $[M+{}^{39}K]^+$ ion also found at m/z 893. The following information can be obtained from FD-MS. (1) The presence of two signals at 745 and 731 signifies the loss of arabinose and rhamnose units from the $[M+Na]^+$ cluster by the protonation mechanism, described in the literature thus assigning the loss to that of the terminal sugars. The former signal at m/z 745 is more intense than the latter at m/z 731, which may be due to the furanose

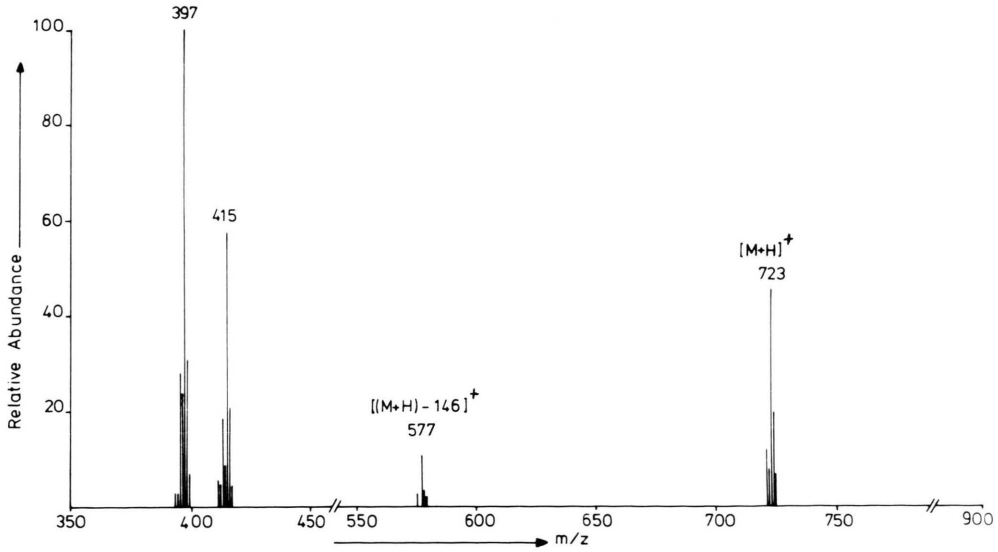

Fig. 2. FAB mass spectrum of polyphyllin C.

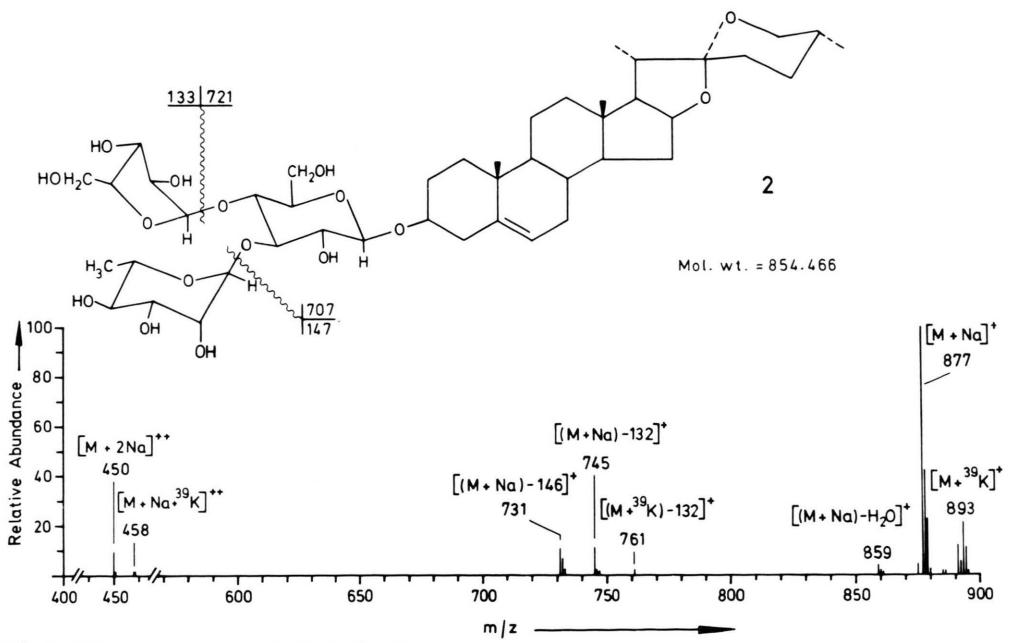

Fig. 3. FD mass spectrum of polyphyllin D.

structure of arabinose being more susceptible to elimination than the pyranose structure of the rhamnose. This faster elimination of furanose sugar is also evident from acidic hydrolysis [19, 20]. The signal at m/z 761 shows the loss of arabinose from the $[M+{}^{39}K]^+$ cluster, again via proton transfer mechanism. The ion due to the loss of the trisaccharide unit was not found in the FD-MS. Under the experimental conditions employed for *soft* ionization, the loss of water from the saponin gave only a weak $[(M+Na)-H_2O]^+$ signal at m/z 859.

The presence of a doubly-charged ion at m/z 450 for $[M+2Na]^{2+}$ is in good agreement with the assignment of the molecular weight of the saponin.

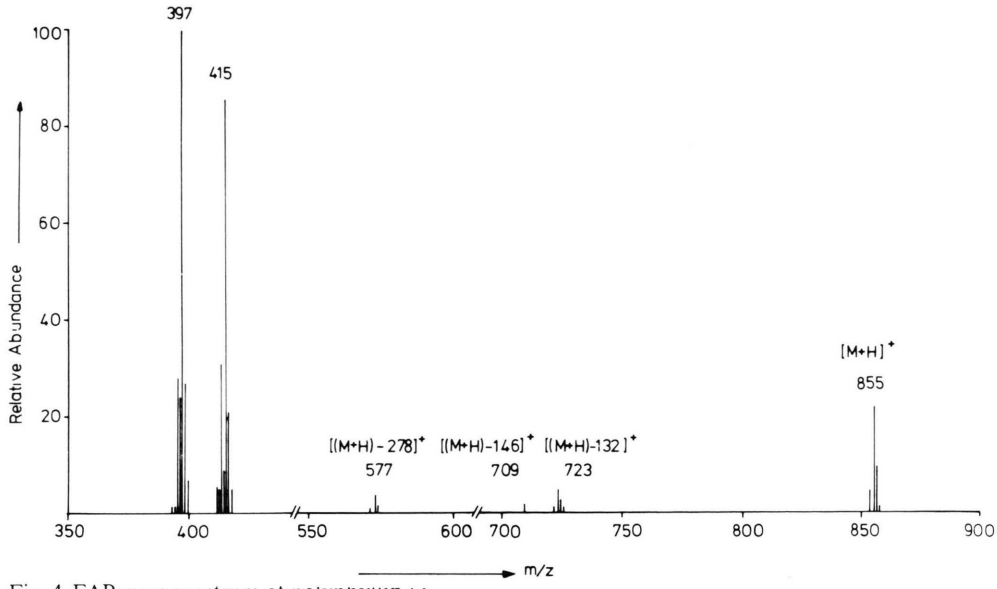

Fig. 4. FAB mass spectrum of polyphyllin D.

Another doubly-charged fragment also is present in this FD spectrum at *m/z* 458 and can be assigned to the attachment of one Na^+ and one $^{39}K^+$ to the intact molecule, *e.g.* $[M+Na+{}^{39}K]^{2+}$.

Using FAB-MS, the spectrum in Fig. 4 is obtained. Similar to **1**, the FAB process generates very intense ions for the protonated aglycone at *m/z* 415 and its water elimination (base peak at *m/z* 397). Although the abundance is relatively low, the characteristic signals for molecular weight and sugar sequence are detected as demonstrated above for FD-MS. Nevertheless, protonation again prevails, as can be derived from the $[M+H]^+$ ion at *m/z* 855 and the losses for the arabinosyl (*m/z* 723) and rhamnosyl (*m/z* 709) moieties. Obviously, one difference between FD and FAB is that the cleavage at the glycosidic oxygen at C-3 of the sapogenin is observed in FAB but not in FD. On the other hand, doubly-charged molecular clusters are generally prominent in FD-MS but were not detected with FAB.

Polyphyllin E

FD-MS of polyphyllin E (diosgenin-3-O-α-L-rhamnopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 4)[α-L-rhamnopyranosyl(1 → 3)]-β-D-glucopyranoside **3** shows the base peak for the $[M+Na]^+$ ion at *m/z* 1037 and an intense ion for $[M+{}^{39}K]^+$ at *m/z* 1053 (Figure 5).

The signals at *m/z* 891 for $[(M+Na)-146]^+$ and *m/z* 907 for $[(M+{}^{39}K)-146]^+$ indicate the loss of one of the two terminal rhamnosyl residues. This direct bond cleavage together with hydrogen transfer, is similar to the fragmentation pathway reported for FD-MS of oligosaccharides [21, 22]. The ions at *m/z* 745 for $[(M+Na)-292]^+$ and *m/z* 761 for $[(M+{}^{39}K)-292]^+$ are produced by the fission of a dirhamnosyl residue of the molecule accompanied by hydrogen transfer. These signals can be obtained also by the loss of both terminal residues, which is less probable although such a process cannot be excluded and would give isomeric ions as end products. In addition, a signal of 12% relative abundance is found at *m/z* 599 which clearly indicates the loss of all three terminal rhamnose moieties. As observed in the FD spectra of compounds **1** and **2** the fission at C-3 of the aglycone and loss of the complete glycosidic chain is disfavoured. The corresponding signal, however, which gives the information for the aglycone, is detected with 5% relative abundance at *m/z* 437 when higher emitter heating currents are employed. Also it has been possible to find the doubly-charged ion clusters at *m/z* 530 for $[M+2Na]^{2+}$ and *m/z* 538 for $[M+Na+{}^{39}K]^{2+}$ which support the correct assignment of the molecular weight. In this FD mass

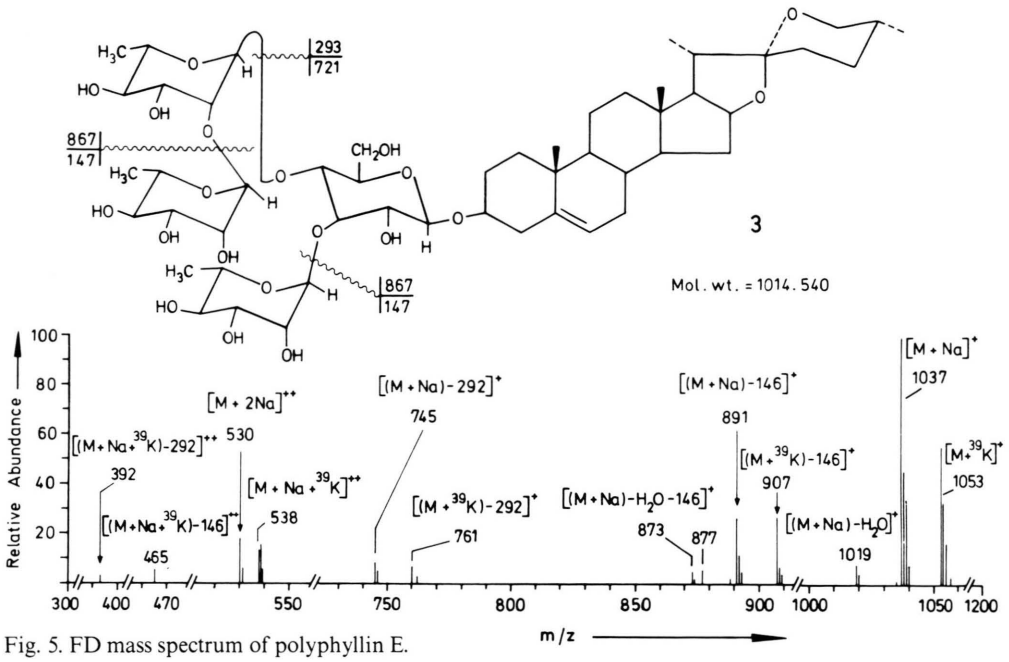

Fig. 5. FD mass spectrum of polyphyllin E.

spectrum are some other doubly-charged ions which also were obtained due to the loss of sugar units *viz.* *m/z* 465 $[(M+Na+^{39}K)-146]^{2+}$ and *m/z* 393 for $[(M+Na+^{39}K)-292]^{2+}$. With these results FD-MS is thus in complete agreement with the correct assignment of the molecular weight of **3** as well as providing useful information about its structure. FAB-MS of this compound gives information similar to that described above for **1** and **2**.

Polyphyllin F

Investigations of polyphyllin F (diosgenin-3-α-L-rhamnopyranosyl(1 → 4)[α-L-rhamnopyranosyl(1 → 3)][β-D-glucopyranosyl(1 → 2)]α-L-rhamnopyranoside, **4**) by FD-MS (Fig. 6) show very intense ion formation due to association of potassium cations instead of Na^+. This deviation from the normal situation is probably due to the fact that this compound was obtained by alkaline hydrolysis with potassium hydroxide of the corresponding acetate. As usual, the molecular cluster at *m/z* 1037 for $[M+Na]^+$ and *m/z* 1053 for $[M+^{39}K]^+$ is obtained in the FD spectrum, but in this case the latter quasi-molecular peak is more intense (base peak) than the former. Fragments are obtained at *m/z* 891 for $[(M+Na)-146]^+$ and/or $[(M+^{39}K)-162]^+$, *m/z* 907 for $[(M+^{39}K)-146]^+$, resulting from the loss of a monorhamnosyl residue and at *m/z* 875 $[(M+Na)-162]^+$, due to loss of the glucose residue. The fragments generated by the loss of two rhamnosyl residues are also present at *m/z* 745 for $[(M+Na)-292]^+$ and *m/z* 761 for $[(M+^{39}K)-292]^+$. In this case, these signals were certainly due to cleavage of the two terminal rhamnose units, probably in the same way as the FD-MS of polyphyllin E. Ions at *m/z* 599 and 583 also are produced which can be explained by $[(M+^{39}K)-2\times 146-162]^+$ and $[(M+Na)-2\times 146-162]^+$ signals. These are formed by the loss of all terminal monosaccharides leaving diosgenin mono-rhamnoside as residue. In this manner the attachment of rhamnose to the diosgenin at C-3 is confirmed. This type of signal is similar to that in the FD of **3**. The aglycone ion was obtained by a very weak signal at *m/z* 421 due to cleavage of the molecule at C-3. Hydrogen transfer and subsequent Na^+ attachment gives the $[(M+Na)-616]^+$ ion. Furthermore, the $[(M+H)-616]^+$ ion is observed at *m/z* 399 and clearly indicates the protonated aglycone. The doubly-charged ions were obtained in this case at *m/z* 530 for $[M+2Na]^{2+}$ and *m/z* 538 for $[M+Na+^{39}K]^{2+}$, again the latter ion peak was more intense (base peak) than the former. This once

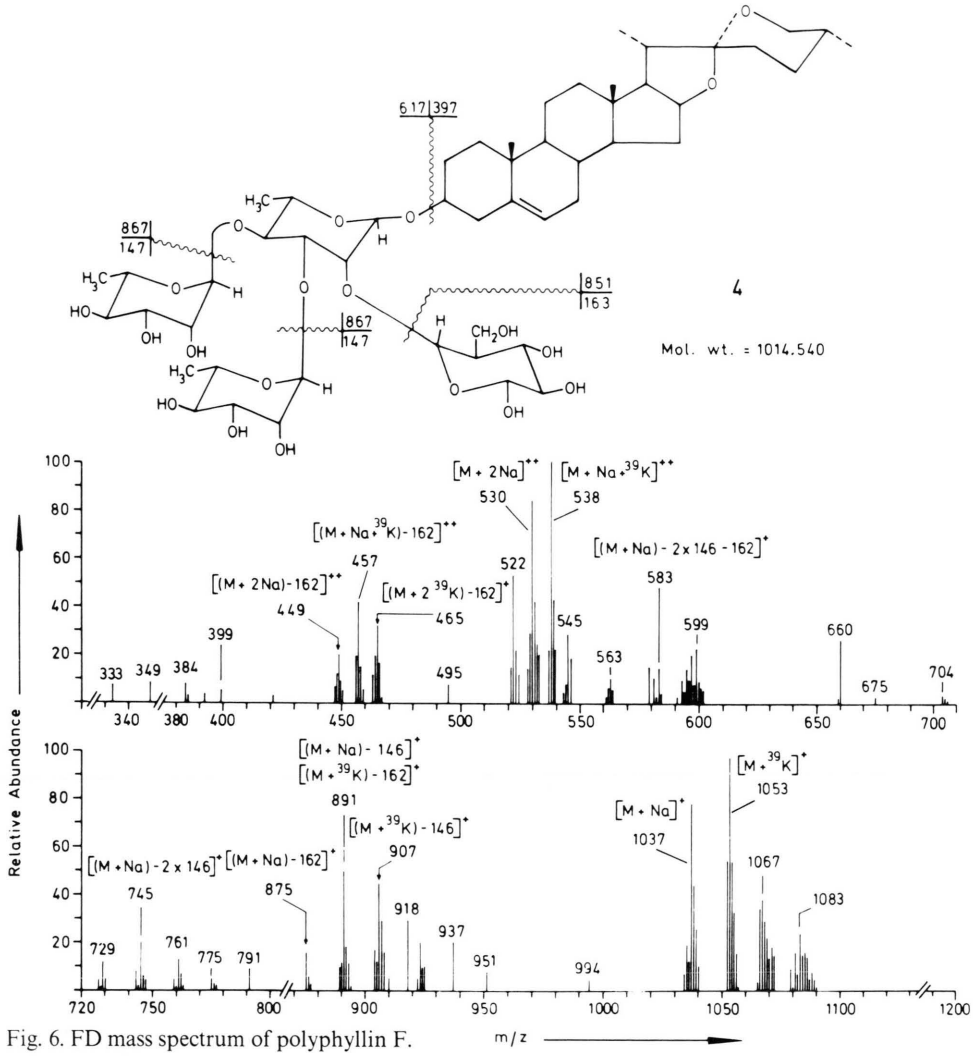

Fig. 6. FD mass spectrum of polyphyllin F.

again supports the correct assignment of the molecular weight. Other doubly-charged ions were also present as in the FD spectrum of **3** at m/z 457 for $[(M+2Na)-146]^{2+}$, m/z 465 for $[(M+Na+{}^{39}K)-146]^{2+}$ and at m/z 392 for $[(M+Na+{}^{39}K)-292]^{2+}$ and at m/z 384 for the $[(M+2Na)-292]^{2+}$ ion. At higher emitter heating currents (30–32 mA), a second saponin with mol. wt. 1060 was detected in this specimen of **4**. Initially, this accompanying substance, with an $[M+Na]^+$ ion at 1083, looses methanol to give m/z 1051. The loss of one (m/z 937) and two (m/z 791) rhamnosyl residues is observed and, in addition, cleavage of a terminal hexose at m/z 921. Since the $[M+2Na]^{2+}$ ion at m/z 553 is clearly discerned, the presence of this minor admixture is reliably detected and demonstrates the potential of FD-MS for purity control.

Polyphyllin G

Polyphyllin G (3-O-{-α-L-rhamnopyranosyl(1 → 3)-[α-L-arabinofuranosyl(1 → 4)]-β-D-glucopyranosyl} 26-O-[β-D-glucopyranosyl]-(25R)-22-α-methoxy-furost-5, en-3β, 26-diol, **5**) is a furostanol saponin. The FD mass spectrum of this compound in Fig. 7 shows that the Na^+ and K^+ ions are attached, as usual, to the complete molecule as well as to its fragments. The $[M+Na]^+$ ion at m/z 1071 is not obtained as base peak. The relatively low abundance of this peak is due to the very easy elimina-

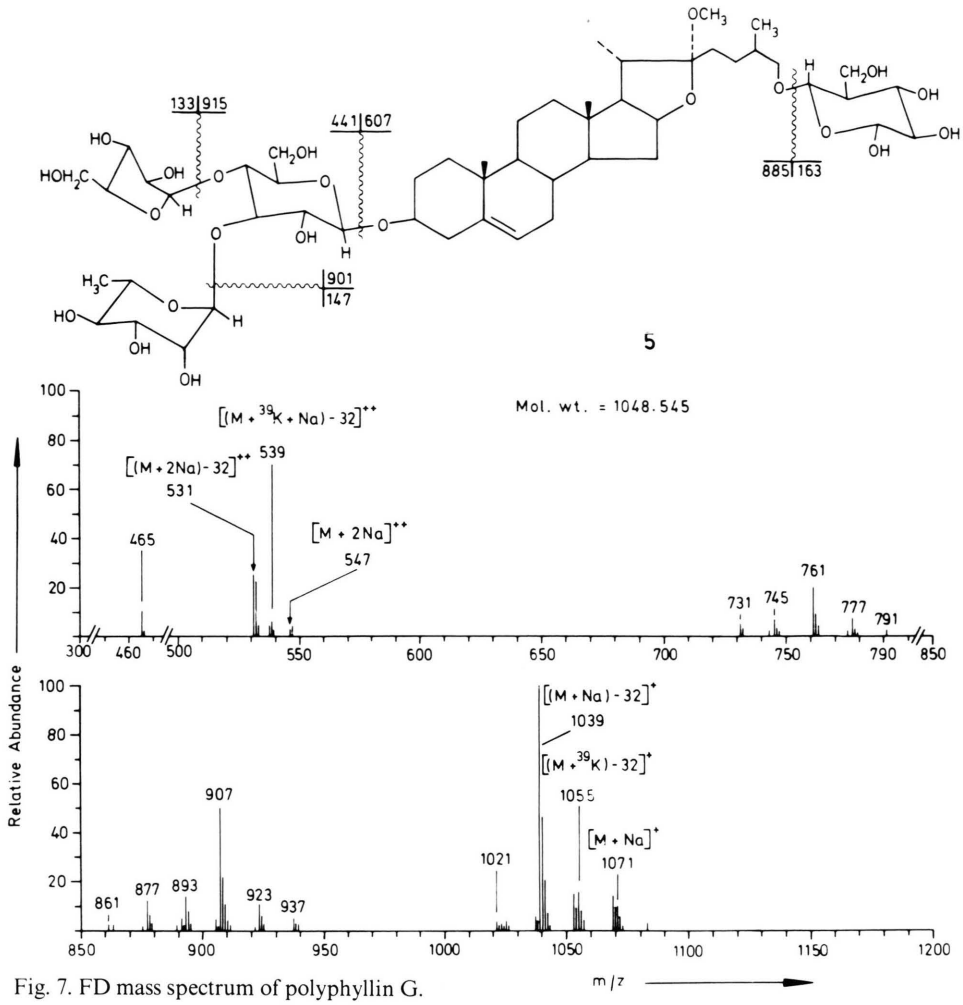

Fig. 7. FD mass spectrum of polyphyllin G.

tion of MeOH from the 22-α-position of the sapogenin, producing a base peak at *m/z* 1039 corresponding to $[(M+Na)-MeOH]^+$ ion. This type of loss of MeOH or EtOH from the 22-α-position is a very feasible elimination in EI-MS of the methylated as well as acetylated derivates of furostanol saponins, in which case, $M^{+\cdot}$ ions did not appear at all [5]. Thus, in FD-MS of pennogenin glycosides, the elimination of H_2O from the 17-OH group was responsible for producing the base peak for the $[(M+H)-H_2O]^+$ signal rather than $[M+H]^+$ [8]. The assignment of molecular weight 1048 is supported also by the presence of a signal for $[M+2Na]^{2+}$ at *m/z* 547. Other doubly-charged ions are also present at *m/z* 531, and 539 corresponding to $[(M+2Na)-MeOH]^{2+}$ and $[(M+Na+^{39}K)-MeOH]^{2+}$ respectively; these too are in close agreement with the assigned molecular weight. The following structural information has been obtained from the other fragments: (1) An intense fragment is obtained at *m/z* 907 due to the loss of 132 mass units from the $[(M+Na)-MeOH]^+$ ion via the described protonation process, which suggests that arabinose is a terminal sugar. The very high intensity of this signal with respect to other sugar losses also gives some information regarding the furanose structure of the arabinose. Since the sugars having furanose structure are more easily hydrolysable than the pyranose sugars, this also supports the above observation. The cleavage mechanism for the glyco-

sidic linkage of the sugars in FD-MS is almost same as that in the acid solvolysis which proceeds by protonation as described recently by Komori *et al.* [9]. The signal at *m/z* 923 is due to $^{39}K^+$ attachment *i.g.* $[(M+{}^{39}K) - MeOH - 132]^+$.

(2) The cleavage of the molecule **5**, losing one terminal rhamnose and producing a peak at *m/z* 893 corresponding to ion $[(M+Na) - MeOH - 146]^+$.

(3) Loss of 162 units from the parent fragment furnishes an ion at *m/z* 877 $[(M+Na) - MeOH - 162]^+$ and can be explained by the elimination of a terminal glucose unit attached at C-26. The intensity of this signal is weak as compared to the above two signals, probably due to β-linkage.

(4) Following the elimination of MeOH, the signals obtained by the loss of two sugar units at *m/z* 761, 745 and 731 can be explained by the cleavage of **5** and give $[(M+Na) - MeOH - 278]^+$, $[(M+Na) - MeOH - 294]^+$ and $[(M+Na) - MeOH - 308]^+$ ions, respectively. Similarly weak signals for potassium attachment are detected, *e.g.* *m/z* 777 for the $[(M+{}^{39}K) - MeOH - 278]^+$ ion.

(5) Two weak signals corresponding to mass units 576 and 560 represent the cleavages at C-3 of the sapogenin with and without the adjacent glycosidic oxygen. In the former cleavage, the steroidal moiety is with the glycosidic oxygen as usual reaction, whereas in the latter case, the steroidal moiety looses its bridging oxygen of the ether linkage.

On the basis of above data, the presence of one glucose unit at a position other than C-3 of the aglycone is evident. The only other position available in the diosgenin molecule for glycosidation is C-26. Thus, the glucose unit must be present at C-26 of diosgenin, forming a furostanol derivatives with the methylation of the 22-hydroxy group. It is noteworthy that even after careful purification, the sample still contains traces of other saponins with mol. wts. of 1030 and 1044, which can easily be detected using FD-MS.

In Fig. 8, a survey of the ions generated by FAB-MS of **5** is given. In the recorded mass range between *m/z* 300 and *m/z* 1100, a great plurality of signals is detected. However, taking into account the FD results the interpretation of the FAB mass spectrum is straightforward:

(1) An intense fragment is obtained at *m/z* 885 due to the loss of 132 mass units from the $[(M+H) - MeOH]^+$ ion and this indicates the loss of the terminal arabinose. Although sodium chloride was added to the glycerol/methanol matrix of **5** and an intense $[M+Na]^+$ ion at *m/z* 1071 was

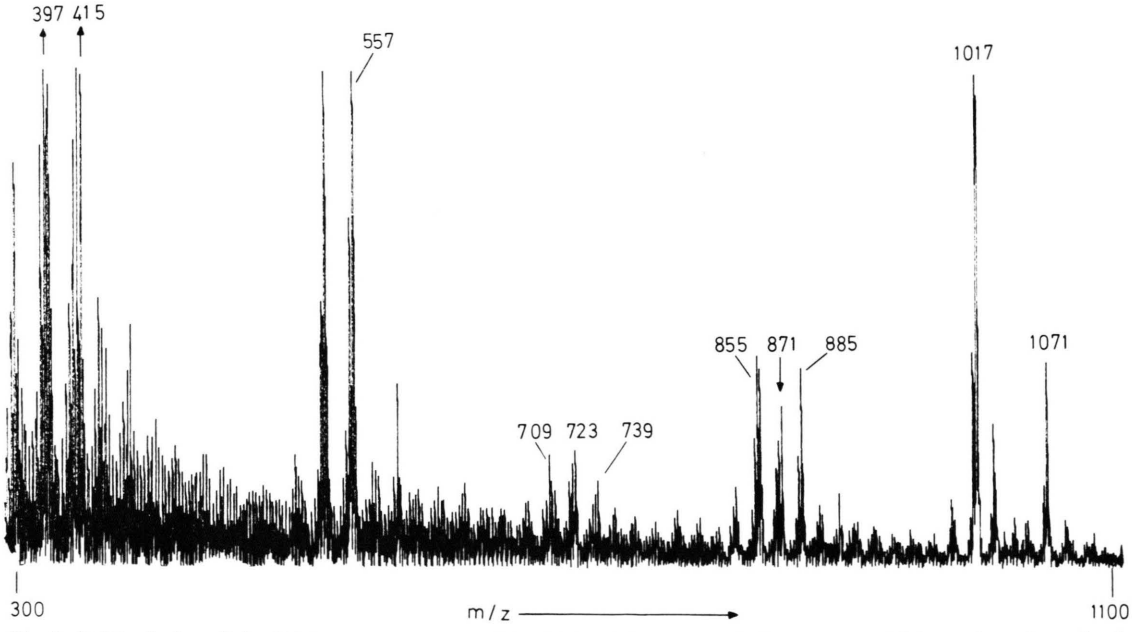

Fig. 8. Original plot of the FAB mass spectrum of **5** obtained by accumulation on a multichannel analyser in the mass range 300 to 1100.

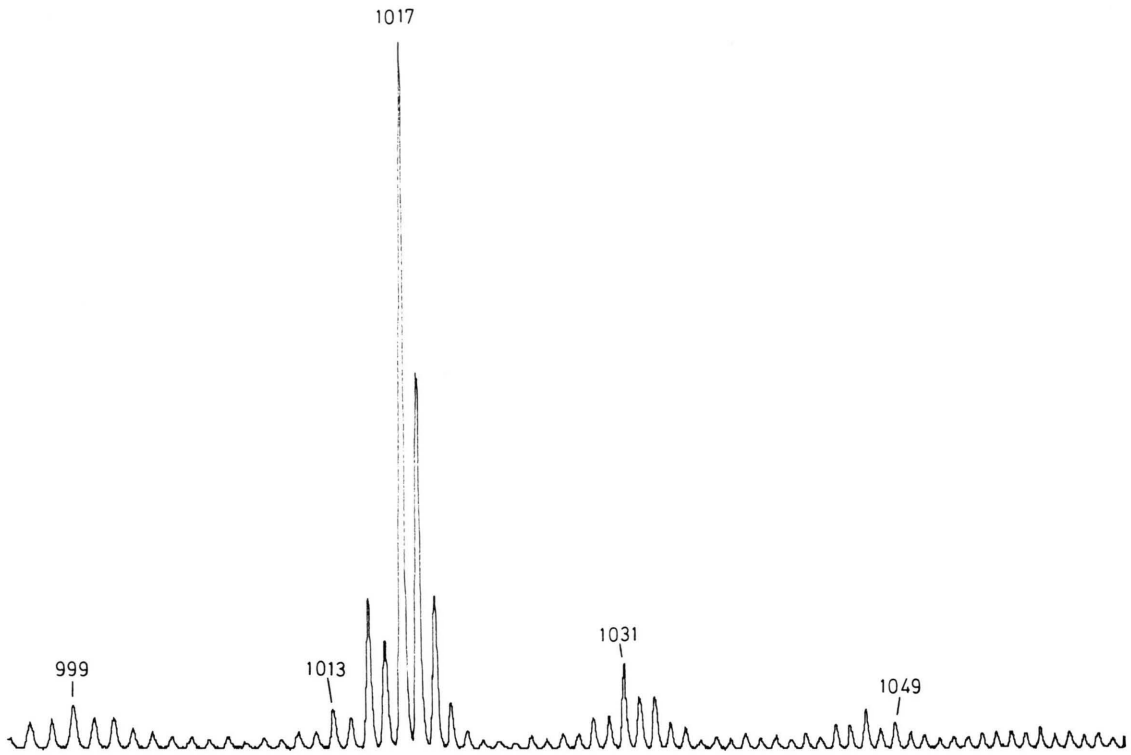

Fig. 9. Original plot of the FAB ions in the molecular ion region of **5**, recording conditions as described for Fig. 8.

detected in this FAB mass spectrum, the sequence-specific signals in the oligosaccharidic chain are derived from the protonated complex after elimination of methanol. The corresponding $[(M+H) - MeOH]^+$ ion is the most abundant type of ion in the mass range above *m/z* 415.

(2) The cleavage of the molecule **5** losing the terminal rhamnose, produces a peak at *m/z* 871 for the $[(M+H) - MeOH - 146]^+$ ion.

(3) Loss of 162 mass units from the parent fragment gives an ion at *m/z* 855 corresponding to elimination of the glucose moiety attached at C-26. As shown in Fig. 8, the relative abundances of these ions for the three terminal sugar cleavages are quite similar in FAB-MS.

(4) Following the elimination of MeOH and protonation of the rearranged molecule, the loss of two sugars can be derived from the $[(M+H)-MeOH-278]^+$, $[(M+H)-MeOH-294]^+$ and $[(M+H) - MeOH - 308]^+$ ions at *m/z* 739, *m/z* 723 and *m/z* 709, respectively. Their intensity, however, is fairly close to the chemical noise level produced in the FAB process and their assignment for an unknown compound would be difficult.

(5) In contrast to FD-MS, the FAB mass spectrum shows very intense signals generated by cleavages at the C-3 of the sapogenin with and without the adjacent glycosidic oxygen. This loss of the trisaccharidic chain is clearly revealed at *m/z* 577 and *m/z* 561. Furthermore, very high abundances for the aglycone and ist product after water elimination are shown at *m/z* 415 and 397, respectively. This observation is consistent with the results described above for compounds **1**–**3**.

In order to confirm the molecular weight determined by FD-MS, it was of interest to have a closer look at the molecular region of **5** by FAB. As shown in Fig. 9, selection of a smaller mass range (*m/z* 997 to *m/z* 1054), repetitive magnetic scanning and recording of the FAB ions on a multichannel analyser gives a simple overview. The prominent ion is the $[(M+H) - MeOH]^+$ at *m/z* 1017, which has been described above as starting point for sugar sequencing. The $[M+H]^+$ ion at *m/z* 1049 is just

detectable above the noise level. If the accompanying compounds of **5** detected by FD-MS are furostanol saponins (mol. wts. 1030 and 1044), their products after MeOH elimination could be derived from the ions at m/z 999 and m/z 1013.

In summarizing these preliminary results of FD- and FAB-MS of saponins, it appears that the *dilution* of sample molecules in the glycerol matrix is a crucial effect. The results are firstly, a reduction of the influence of surfaces secondly, reduction of bimolecular (or biionic) reactions and thirdly, sample supply to the actual target area by diffusion and thermal convection. Whereas in FD-MS of saponins multiply-charged ions are generally prominent, for instance $[M+2Na]^{2+}$, this type of ion could not be detected by FAB.

Experimental

The FD and FAB measurements were performed on a type 731 Finnigan MAT double-focusing mass spectrometer equipped with a combined commercial EI/FD/FAB ion source.

For FD-MS the emitters used were 10 μm tungsten wires activated at high temperature. The length of the carbon microneedles was 40 μm on average. The molecular ion of acetone (m/z 58) was used for adjustment and calibration of the emitter. All spectra were produced using direct heating of the emitter by a heating current. The sample was transferred to the emitter by the modified syringe technique and in general 1 to 3 μg of sample material were deposited on the center of the front side of the emitter. The applied potentials were +8 kV for the field anode and −4 kV for the opposing cathode plate. The FD ion currents were recorded electrically in combination with the Finnigan SS 200 data system. All spectra were acquired at a mass resolution of about 2000 (10% valley definition).

For FAB-MS the instrument was equipped with a FAB pushrod introduction system and a saddle field ion gun and power supply (Ion Tech Ltd., Teddington, England) modified by Finnigan MAT for neutral beam production. The atom gun was mounted on the laser port of the ion source housing [23] resulting in an atom beam perpendicular to the beam of FAB ions leaving the ion source. Xenon of a purity ≧ 99.99 vol% (Messer Griesheim, Düsseldorf, FRG, article nr. 816078) was used as collision gas. The Xe^+ current output on average was 0.05 mA. The samples were supplied to the copper target in methanol solution and mixed with glycerol under optical control with a stereomicroscope (magnification ~ × 30). The recording of the FAB ions was as described above for FD-MS with the exceptions of Figs. 8 and 9 which were accumulated on a multichannel analyser (Canberra Industries, Inc., Meriden, CT 06450, USA).

Conclusion

The role of FD-MS in determinations of the molecular weights of the thermally labile saponins [24] is further enhanced by elucidating the sequence of sugar units and also the characteristic behaviour of furastanol saponins. The relative abundances of the peaks suggest the configuration and ring size of the sugar units. Studies of the fragmentation pattern obtained in FD-MS thus assist structural work on saponins.

The additional use of FAB-MS as a confirmatory and supporting method puts our results on a second footing. Unambigeous molecular weight determination and elucidation of the sugar sequence of saponins appears feasible. Although matrix and sample effects play an important role [25], the combination of FD/FAB is thought to be an efficient analytical tool for natural product analyses.

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[1] S. B. Singh and R. S. Thakur, Planta Med. **40,** 301 (1980).
[2] S. B. Singh and R. S. Thakur, Phytochemistry **42,** 911 (1982).
[3] S. B. Singh, R. S. Thakur, and H.-R. Schulten, Phytochemistry **21,** 2925 (1982).
[4] S. B. Singh, R. S. Thakur, and H.-R. Schulten, Phytochemistry **21,** 2079 (1982).
[5] T. Komori, Y. Ida, Y. Mutou, K. Miyahara, T. Nohara, and T. Kawasaki, Biomed. Mass Spectrom. **2,** 65 (1975).
[6] H.-R. Schulten, Int. J. Mass. Spectrom. Ion Phys. **32,** 99 (1979).
[7] H.-R. Schulten, T. Komori, and T. Kawasaki, Tetrahedron **33,** 2595 (1977), and references cited therein.
[8] H.-R. Schulten, T. Komori, T. Nohara, R. Higuchi, and T. Kawasaki, Tetrahedron **34,** 1003 (1978).
[9] T. Komori, I. Maetani, N. Okamura, T. Kawasaki, T. Nohara, and H.-R. Schulten, Liebigs Ann. Chem. **1981,** 683.
[10] H. Wagner, H. Habermeier, A. Liptak, and H.-R. Schulten, Planta Med. **37,** 381 (1979).
[11] H.-R. Schulten, T. Komori, T. Kawasaki, and S. Shibata, Planta Med. **46,** 67 (1982).
[12] K. Kudo, T. Nohara, T. Komori, T. Kawasaki, and H.-R. Schulten, Planta Med. **40,** 250 (1980).
[13] T. Komori, T. Kawasaki, and H.-R. Schulten, Mass Spectrom. Rev., in press.
[14] M. Barber, R. S. Bordoli, R. D. Sedgwick, and A. N. Tyler, J. Chem. Soc. Chem. Commun. **7,** 325 (1981).
[15] M. Barber, S. Bordoli, R. D. Sedgwick, and A. N. Tyler, Nature (London) **293,** 270 (1981).
[16] M. Barber, S. Bordoli, R. D. Sedgwick, L. W. Tetler, and A. N. Tyler, Biochem. J. **197,** 401 (1981).
[17] W. G. Overend, C. W. Rees, and J. S. Sequeira, J. Chem. Soc. **1962,** 3429.
[18] J. N. Bemiller, Advances in Carbohydrate Chemistry (M. L. Wolfrom and R. S. Tipson, eds.), pp. 43, 52, Academic Press, New York 1967.
[19] K. P. Tewari and R. B. Singh, Phytochemistry **17,** 1991 (1978).
[20] J. K. N. Jones and F. Smith, Advances in Carbohydrate Chemistry **Vol. 2,** pp. 243, Academic Press, New York 1946.
[21] J. Moor and E. S. Waight, Org. Mass Spectrom. **9,** 903 (1974).
[22] H.-R. Schulten, Methods of Biochemical Analysis (D. Glick, ed.), **Vol. 24,** pp. 313–448, and references cited therein, Interscience, New York 1977.
[23] H.-R. Schulten, P. B. Monkhouse, and R. Müller, Anal. Chem. **54,** 654 (1982).
[24] T. Komori, M. Kawamura, K. Miyahara, T. Kawasaki, O. Tanaka, S. Yahara, and H.-R. Schulten, Z. Naturforsch. **34c,** 1094 (1979).
[25] W. D. Lehmann, M. Kessler, and W. A. König, Biomed. Mass Spectrom., in press.
[26] K.-W. Glombitza, S. Wegner-Hambloch, and H.-R. Schulten, Planta Med., in press.