Journal of Chromatography, 212 (1981) 37–49
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,806

IDENTIFICATION OF GINSENOSIDES FROM *PANAX GINSENG* IN FRACTIONS OBTAINED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY BY FIELD DESORPTION MASS SPECTROMETRY, MULTIPLE INTERNAL REFLECTION INFRARED SPECTROSCOPY AND THIN-LAYER CHROMATOGRAPHY*

H.-R. SCHULTEN
Institute of Physical Chemistry, University of Bonn, Wegelerstrasse 12, 5300 Bonn (G.F.R.)
and
F. SOLDATI
Research and Development, Pharmaton Ltd., Lugano-Bioggio (Switzerland)
(First received February 26th, 1981; revised manuscript received March 19th, 1981)

SUMMARY

The high-performance liquid chromatographic (HPLC) determination of the ginsenoside saponins from *Panax ginseng* C. A. Meyer (Araliaceae) was used to investigate the possibilities and limitations of peak identification with on-line and off-line methods. Thin-layer chromatography was used to separate the ginsenosides with a running distance of 6.5 cm, the detection limit being 0.2 μg. By means of multiple internal reflection infrared (IR) spectroscopy, IR spectra were obtained with 20 μg of ginsenoside. Field desorption (FD) mass spectrometry permitted not only the identification and determination of the molecular weights of underivatized ginsenosides, but also gave important information about the sequence of the sugar moities in the molecule. Less than 1 μg of ginsenoside is needed to produce an FD spectrum. The time required for the FD investigation of an HPLC fraction containing a ginsenoside is about 1 h, including sample preparation, FD measurements, data processing, output, evaluation and interpretation.

INTRODUCTION

We recently reported on the possibility of separating ginsenosides (triterpene saponins from *Panax ginseng* C.A. Meyer) by means of high-performance liquid chromatography (HPLC) and of determining them quantitatively in plant materials and in various galenical preparations[1,2]. An important aspect, which often is not considered when using HPLC, is the unambiguous and reliable identification of the eluted substances. The examination of the purity of a peak is particularly important if there are substances to be determined in complex mixtures, such as body fluids or

* Field desorption mass spectrometry of natural products, Part IX; for Part VIII, see ref. 12.

0021-9673/81/0000–0000/$02.50

plant extracts. Utilizing the HPLC determination of the ginsenosides, we report here the possibilities and limitations of peak identification using on-line methods such as ultraviolet (UV) spectroscopy and absorbance ratios and off-line methods, such as thin-layer chromatography (TLC), multiple internal reflection infrared (MIR-IR) spectroscopy and field desorption mass spectrometry (FD-MS). The structures of the ginsenosides investigated are shown in Fig. 1.

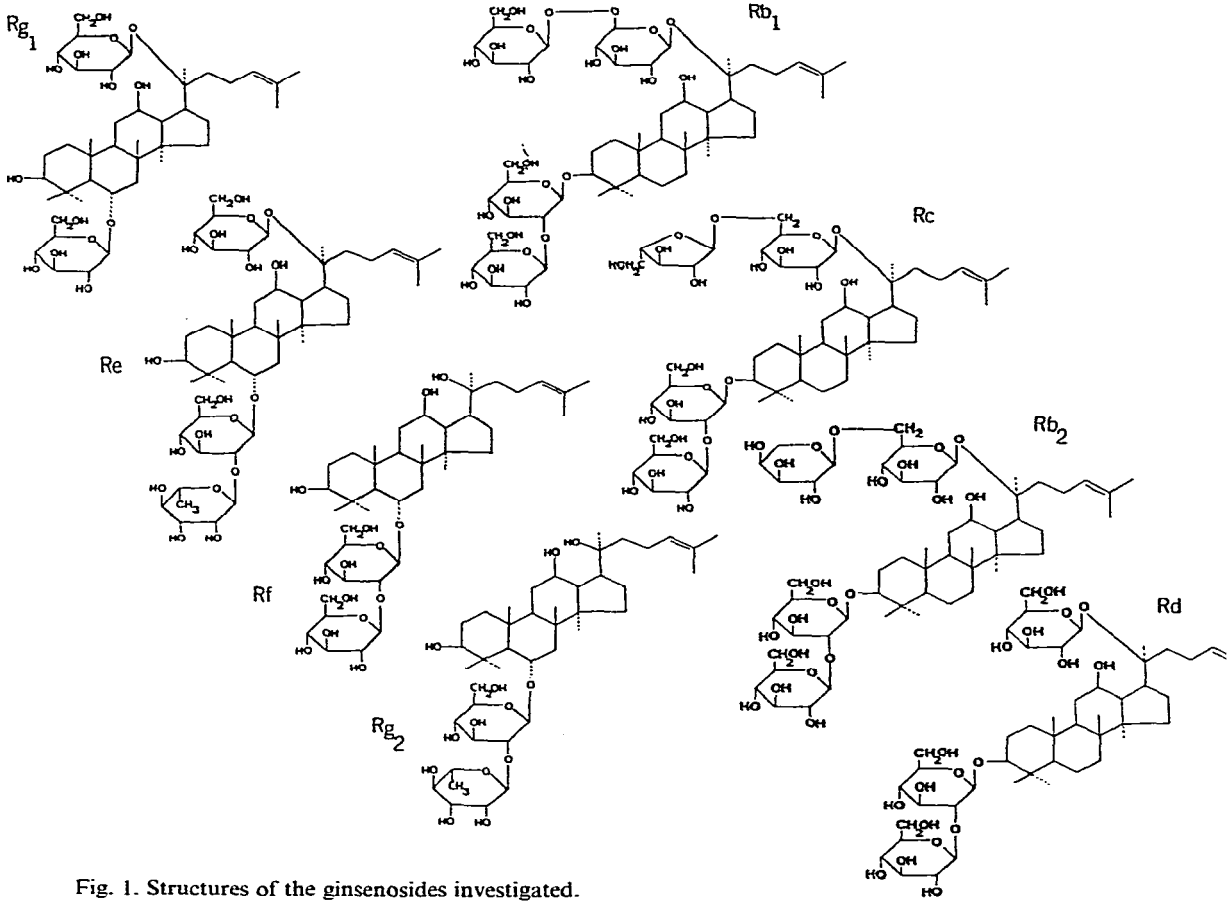

Fig. 1. Structures of the ginsenosides investigated.

EXPERIMENTAL

Apparatus

A Model 6000 A chromatographic pump, a Model U6K sample injection valve and a pre-packed μBondapack C_{18} column (30 cm × 3.9 mm I.D.) were obtained from Waters Assoc. (Milford, MA, U.S.A.), and an LC 75 spectrophotometric detector, Model 598 infrared spectrophotometer and micro MIR accessory from Perkin-Elmer (Norwalk, CT, U.S.A.). An Extrelut column and silica gel HPTLC plates (10 × 10 cm) were purchased from Merck (Darmstadt, G.F.R.) and a Linomat III from Camag (Muttenz, Switzerland). A W + W Series 1100 recorder was supplied by W +

W Electronic (Basle, Switzerland) and a MAT 731 mass spectrometer and Spectro-System 200 by Varian (Bremen, G.F.R.).

Materials

Methanol *n*-butanol and acetonitrile (Uvasol grade), chloroform, acetic anhydride, sulphuric acid and ethanol were Merck products. Perfluorokerosene and tris-(perfluorononyl)-*s*-triazine were obtained from J. T. Baker (Deventer, The Netherlands). Standardized ginseng extracts G115 and G115 S and ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1 and Rg_2 were supplied by Pharmaton (Lugano-Bioggio, Switzerland).

Sample preparation

A 1.0-g amount of ginseng extract was dissolved in 20.0 ml of water in a tared flask and 4.0 ml of this solution and 6.0 ml of water were added to an Extrelut column. After 15 min the column was eluted with 80.0 ml of water-saturated *n*-butanol. The eluate was evaporated on a Rotavapor and the residue dissolved in 2.0 ml of methanol. A 5–30-μl volume of this solution was injected into the HPLC system. The plastic material of new columns must be soaked in methanol for 1 h prior to the first use; before further use washing with common detergents is sufficient. The refilling material must be washed with methanol prior to use (for instance, six bags of refilling material with 1 l of methanol for 6 h in a Soxhlet apparatus, then dryed at 80°C). The column is packed with 11.3 g of refilling material. For further information, see ref. 1.

HPLC

The high-performance liquid chromatograph consisted of a constant-flow pump, a valve-type injector, a UV detector (203 nm, absorbance 0.08) and a strip-chart recorder (0.5 cm/min). The stainless-steel column was packed with reversed-phase material. The mobile phase was acetonitrile–water (29:71) at a flow-rate of 2 ml/min for ginsenosides Rb_1, Rb_2, Rc, Rd, Rf and Rg_2 and acetonitrile–water (18:82) at a flow-rate of 4 ml/min for ginsenosides Re and Rg_1. After each chromatographic run the column was washed with 30 ml of methanol at 4 ml/min. Room temperature (22°C) was used.

TLC

The fractions relating to the peaks of Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1 and Rg_2 were collected from the chromatograph in semi-micro flasks with conical bottoms, then the solvent was evaporated. The residue was dissolved in a few microlitres of methanol and the solution drawn off with a syringe. Pre-coated HPTLC plates were used for the chromatography, with Linomat III spray-on equipment. The sample volume was 10 μl, band length 5 mm, running speed 50 mm/μl, piston speed 10 sec/μl and nitrogen pressure 2 bar. Eluent A (chloroform–*n*-butanol–methanol–water, 20:40:15:20; lower phase) or eluent B (chloroform–methanol–water, 65:35:10; lower phase) can be used as the mobile phase. The eluent front was 6.5 cm for A and 7.5 cm for B. The plate was sprayed with 10 ml of acetic anhydride–sulphuric acid–ethanol (5:5:90) and heated at 105°C for 10 min. The ginsenosides were revealed with brown-violet colouring.

MIR-IR

A microprocessor-controlled IR spectrophotometer was used. The scan time was 8 min, abscissa expansion 56 cm, Slit medium (resolution at 1100 cm^{-1}, 3 cm^{-1}). A micro multiple internal reflection accessory, including a 45° KRS-5 micro crystal (thallium bromide–iodide), 10 mm long × 5 mm high × 0.5 mm thick, was used. To record the spectrum, 10–20 μl of the peak fractions that are to be investigated are placed with a syringe on the crystal and the solvent (methanol) is evaporated in air.

FD-MS

The FD spectra were produced on a commercially available double-focusing mass spectrometer equipped with a combined electron impact/field ionization/FD ion source. All spectra were recorded electrically with scan times between 4 and 8 sec/decade and at a mass resolution of better than 3000 (10% valley definition). Data acquisition and processing were performed using the data system. For mass calibration the electron impact mass spectra of perfluorokerosene and tris(perfluorononyl)-*s*-triazine were taken. The FD emitters used in all experiments were prepared by high-temperature activation of 10-μm diameter tungsten wires in a home-built multiple activation chamber. In general, emitters with an average length of 30 μm for the carbon microneedles were used and their ionization efficiency and adjustment were determined by means of m/z 58 of acetone in the field ionization mode. All spectra were produced at ion source potentials of +8 kV for the field anode and −3 kV for the slotted cathode plate, an ion source pressure of *ca.* 10^{-7} Torr and an ion source temperature between 50 and 60°C. The samples were desorbed by direct heating using the supplied emitter heating current (0–70 mA) and by controlling the emission of FD ions roughly by the total ion monitor. Methanol was used as solvent for all HPLC fractions and an estimated amount of *ca.* 1 μg was transferred to the emitter by the syringe technique. The time required for the FD investigation of an HPLC fraction containing a saponin was about 1 h, including sample preparation, FD measurements, data processing, output, evaluation and interpretation.

RESULTS AND DISCUSSION

On-line methods

In general, the identification and determination of the substances separated by chromatography can be performed on-line, that is at the same time as the chromatographic separation is achieved. Alternatively, an off-line investigation can be applied, involving collection of the effluent and consecutive examination of the chromatographically isolated fractions by physical or chemical methods. The on-line methods can be characterised as follows.

Retention time. Identical capacity factors of a reference substance and the substance to be determined in the test material is a unreliable peak identification method, as several substances can show identical retention times.

UV spectroscopy. If a UV spectrophotometer is used as a detector, it is possible to record the UV spectrum of substances in the stop-flow mode. In this method, the flow of the mobile phase is stopped when a substance that we wish to identify is in the absorption cell. One must work either with a double-beam spectrophotometer or a single-beam spectrophotometer with a background corrector. Although this peak

identification method has proved effective in certain instances[3,4], it is not suitable when there are two or more substances with identical UV spectra under the same peak. Owing to the C_{24}–C_{25} double bonding, all ginsenosides have identical UV spectra ($\lambda_{max.}$ 203 nm) and molar absorptivities (log ε = 4.66). Therefore, if two or more ginsenosides lie under the same peak, it would not be possible to distinguish them.

Absorbance ratios. Another technique for confirming the purity of compounds that appear within a single peak is to use the absorbance ratios at several UV wavelengths[5]. The flow of the mobile phase is stopped when a substance is in the absorption cell and the absorption at several wavelengths is measured. The ratios of these absorbances give an indication of the spectral properties of the compound in the cell. This technique is also useful for confirming the purity of compounds. It is not suitable when unseparated substances have the same UV spectra and identical molar absorptivities.

Off-line methods

For the off-line methods, which were preferred in this work, the following techniques were employed. After previous treatment the extracts were injected into the high-performance liquid chromatograph; Fig. 2 shows typical chromatograms. The steps in the possible off-line methods for peak identification are shown in Fig. 3. The fractions corresponding to peaks Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1 and Rg_2 are collected from the chromatograph in semi-micro flasks with conical bottoms (step 1). In step 2 the solvent (acetonitrile–water) is evaporated. The residue is then dissolved in a few microlitres of methanol and drawn off with a syringe (step 3).

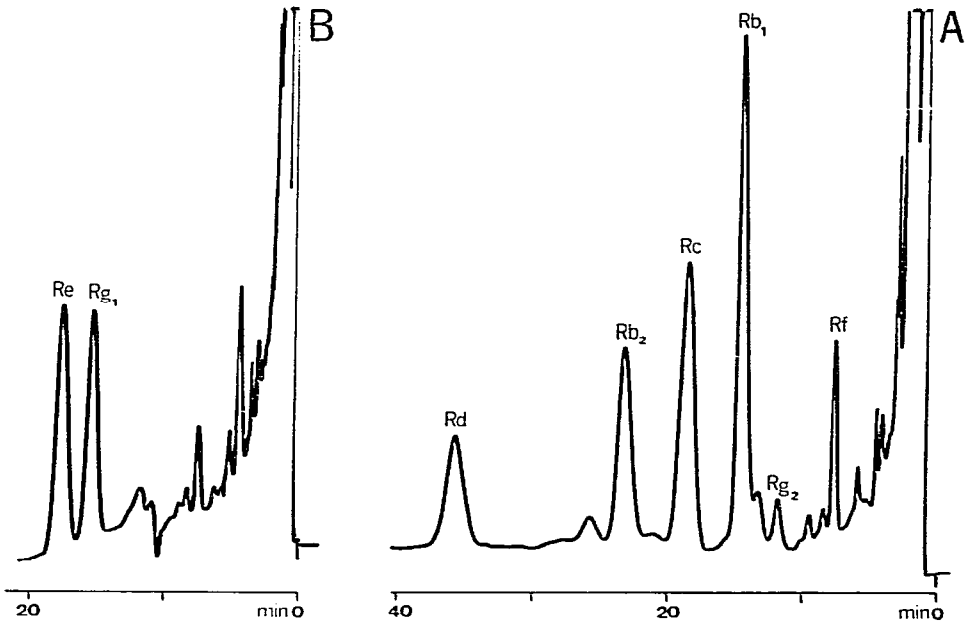

Fig. 2. High-performance liquid chromatograms of G 115 S, 7 μl for eluent A, 12 μl for eluent B. Eluent A: acetonitrile–water (29:71), 2 ml/min. Eluent B: acetonitrile–water (18:82), 4 ml/min. Rb_1, 13 μg; Rb_2, 8 μg; Rc, 11 μg; Rd, 4.4 μg; Re, 17 μg; Rf, 2 μg; Rg_1, 11 μg; Rg_2, 1 μg.

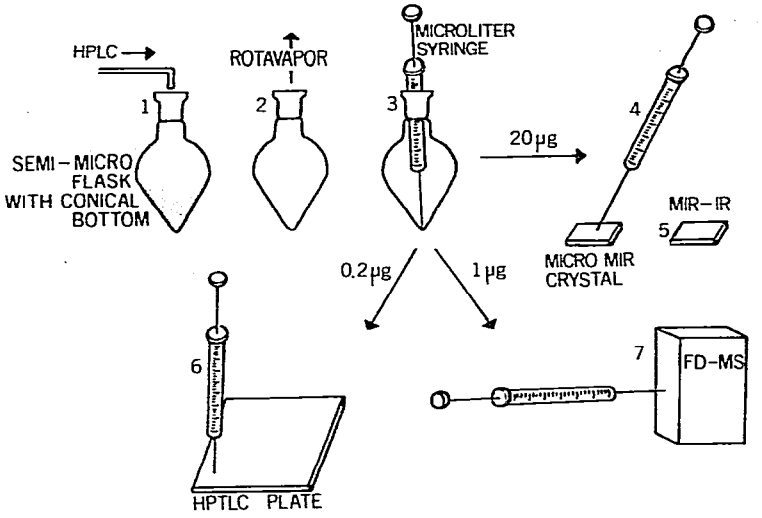

Fig. 3. Off-line methods for identifying substances separated by HPLC.

TLC. Chromatography with HPTLC (step 6) has considerable advantages over classical TLC[6]. For good resolution, it is important to have the minimal diffusion of the spot over as small an area as possible. This is achieved by using a small sample volume and a suitable non-polar solvent. Therefore, water has to be removed from the solvent (step 2).

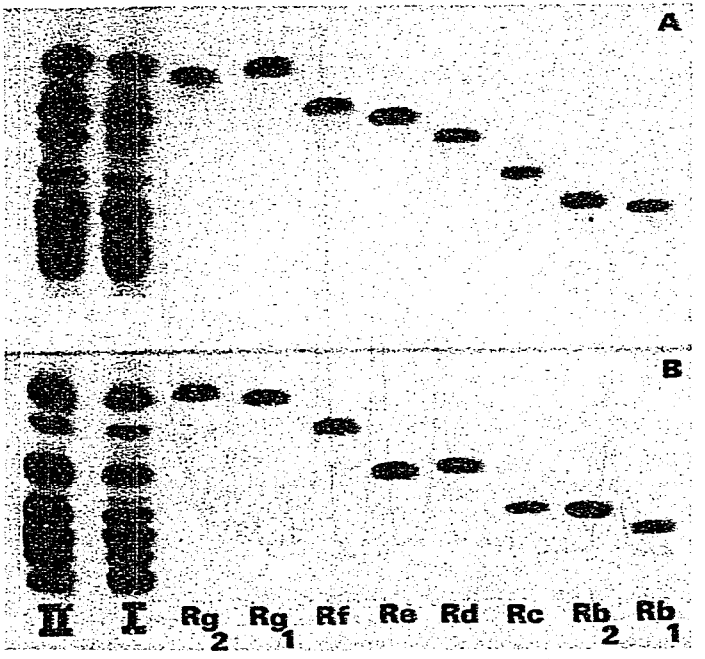

Fig. 4. Thin-layer chromatograms of G115 (I) (10 μl), G115 S (II) (10 μl) and of the peaks Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2 (10 μg each) after HPLC. For A and B, see Experimental.

It is of advantage to combine HPTLC plates with the Linomat spray-on technique, in which nitrogen is used as a carrier to spot the sample on the plate. The free selection of the sample volume between 1 and 100 μl, the accuracy of sample volume sprayed (better than 1%) and the freely selectable band length ensure the highest possible resolution within the shortest time. The results obtained with the HPLC spray-on technique are shown in Fig. 4. The ginsenosides can be separated with a running distance of 6.5 cm with eluent A within 60 min. To carry out an accurate peak identification, it is also possible to chromatograph with other eluents (for instance, B). Table I gives the R_F values obtained. This technique has a detection limit of 0.2 μg of ginsenoside.

TABLE I

RETENTION TIMES (R_F VALUES) OF THE GINSENOSIDES INVESTIGATED BY HPTLC

Ginsenoside	*Eluent A*	*Eluent B*
Rg_2	0.52	0.40
Rg_1	0.53	0.39
Rf	0.44	0.34
Re	0.42	0.25
Rd	0.37	0.26
Rc	0.28	0.23
Rb_2	0.22	0.23
Rb_1	0.21	0.14

MIR-IR spectroscopy. A useful application of MIR-IR spectroscopy is in the identification of peaks separated by HPLC (steps 4 and 5). Typical fraction sizes are 5–20 μg and, after evaporation of the solvent, they can easily be run as films cast on a micro MIR crystal. Total internal reflection occurs within a crystal of high refractive index, along the crystal–sample interface, when energy is passed through it at an angle of incidence greater than the critical angle. At each reflection energy penetrates the sample, which must be in optical contact with the crystal, and a small amount is absorbed at the characteristic absorption frequencies of the sample, producing an IR spectrum[5]. This technique allows the recording of IR spectra with very small amounts of substance. Spectra that we obtained with 20 μg each of the peak fraction corresponding to Rg_1 (A) and Re (B) are illustrated in Fig. 5. In these spectra the absorption bands at 1640 cm^{-1} (C=C) and at 3380 cm^{-1} (OH) are also characteristic for the other ginsenosides.

FD-MS. Gas chromatography–electron impact MS (GC–EI-MS) has been shown to be very useful for the identification and determination of the purity and structures of ginsenosides[7]. However, for the MS investigation volatile derivatives (peracetates or permethyl ethers) have to be produced. Moreover, the ginsenosides do not give molecular ions even when derivatized and even when the spectrum is taken at a low ionizing potential (20 eV)[8].

Previous investigations of natural products by FD-MS have demonstrated that the technique is suitable for the determination of the molecular weights of large, polar and underivatized glycosides. Steroid and triterpene saponins[9], physiologically active pennogenin and hederagenin glycosides[10], cytotoxic cardiac glycosides from *Lopho-*

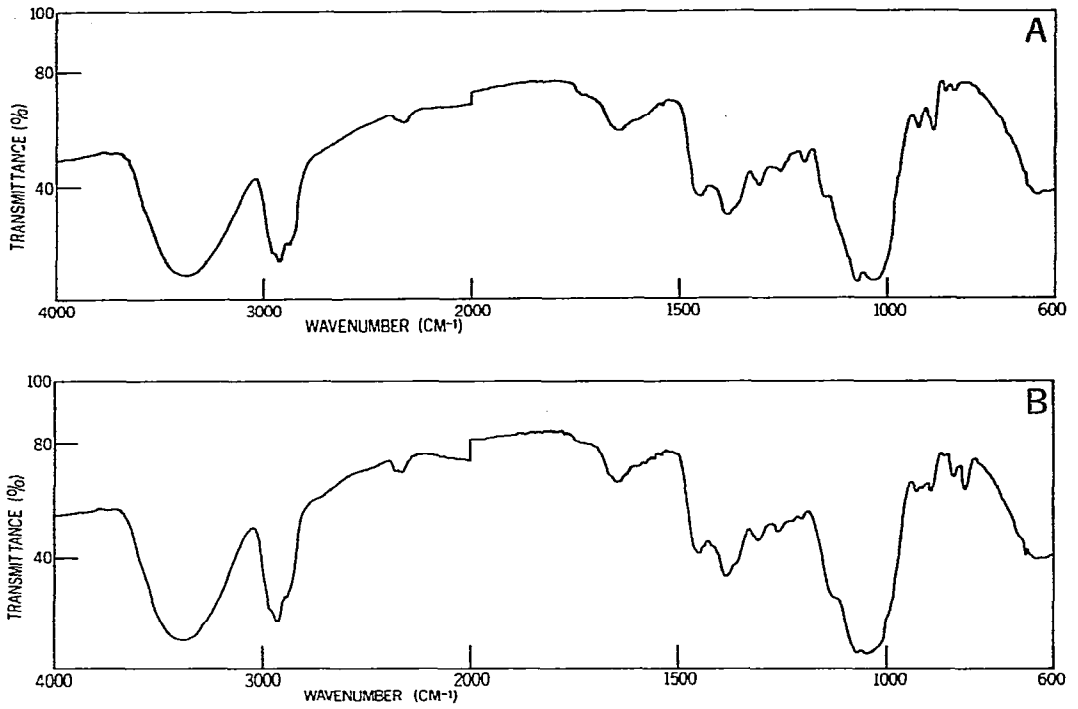

Fig. 5. MIR-IR spectra of the peaks Rg_1 (20 μg) (A) and Re (20 μg) (B) after HPLC.

petalum toxicum[11], lignan glycosides from the barks of *Ligstrum japonicum*[12] and some dammarane saponins[13] have been identified in extracts from plant materials. Moreover, the sequencing of underivatized oligoglycosides and the comparison of sequence-specific fragmentation with the mechanism of solvolysis in solution chemistry have been reported[14]. On the other hand, the utility of FD-MS for the identification of biocides[15–17] and drugs[18–20] in fractions of HPLC effluents, and thus the use of the FD spectrometer as a highly sensitive and specific "off-line" detector, have been amongst the most successful applications of the technique. These results prompted the present investigation to identify and characterize saponins from *Panax ginseng* extracts in HPLC fractions (step 7). The question was whether the FD method allows the rapid and reliable identification and, to some extent, the quality control of physiologically active natural products. In general, all of the ginsenosides investigated gave the $[M + Na]^+$ ion as base peak of the FD mass spectrum, and from this basic feature the molecular weight was easily derived. The compounds Rb_2, Re and Rg_1 had already been studied by FD[13] and the samples from the HPLC fractions gave similar results. For instance, Re gave the $[M + Na]^+$ ion at m/z 969 as base peak (6000 counts), a weak $[M + {}^{39}K]^+$ ion at m/z 985, the $[(M + Na) - H_2O]^+$ ion at m/z 951 (32% relative abundance), and a doubly charged $[M + 2Na]^{2+}$ ion at m/z 496 (15% relative abundance). All of these ions can be used to confirm the correct assignment of the molecular weight and, as will be shown below, are common in the FD spectra of the oligoglycosides. In addition, the loss of a terminal desoxyhexose, rhamnose, is indicated by the $[(M + Na) - 146]^+$ ion at m/z 823 (40% relative

abundance). Further, the loss of a terminal hexose, glucose, results in a weaker signal at m/z 807 for the $[(M + Na) - 162]^+$ ion (22% relative abundance). No other FD ions were detected when eight FD spectra were averaged at emitter heating currents of between 30 and 35 mA.

When six FD spectra of compound Rb_1 were taken between emitter heating currents of 35 and 40 mA the base peak was again the $[M + Na]^+$ ion at m/z 1131 (70,384 counts) and the $[M + 2Na]^{2+}$ ion at m/z 577 was of 12% relative abundance. The sequence-specific ions for the loss of one glucosyl unit, $[(M + Na) - 162]^+$, at m/z 969 and for the loss of the disaccharide consisting of two glucose units at m/z 807, $[(M + Na) - 324]^+$, were of minor importance under these experimental conditions (below 10% relative abundance). Also, compound Rg_1 gave an abundant ion at m/z 807 for $[M + Na]^+$, at m/z 823 for $[M + {}^{39}K]^+$ and m/z 415 for $[M + 2Na]^{2+}$. At the applied emitter heating current of 30–35 mA no sequence-specific fragmentation was induced.

In order to survey which thermally induced fragmentation can be generated and how these fragments can be explained, the ginsenosides Rf, Rd and Rc were investigated in that section of the desorption process which gave the most intense ion currents for structurally significant fragments.

The FD mass spectrum of Rf obtained in this manner is shown in Fig. 6. As mentioned above, the determination of the molecular weight is straightforward as the signal at m/z 823 produced by attachment of a sodium cation to the intact molecule (cationization) gives the base peak of the spectrum. Again, owing to small inorganic

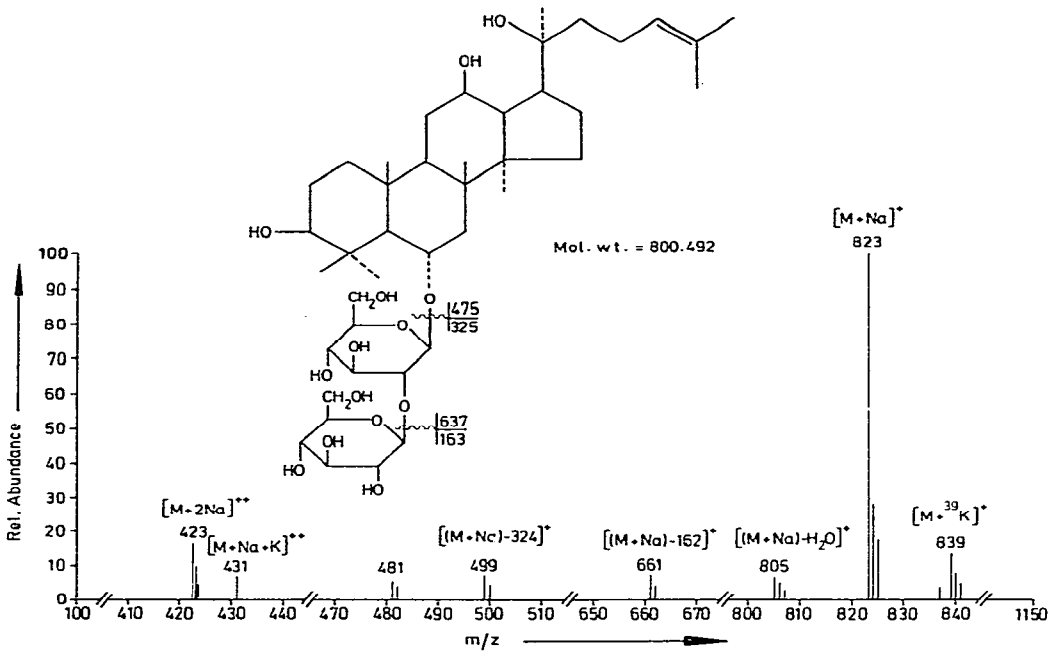

Fig. 6. FD mass spectrum of the HPLC fraction containing compound Rf. Seven mass spectra which were obtained between emitter heating currents of 29 and 32 mA were averaged by the data system. The base peak corresponds to 14,536 counts; the noise level is about 50 counts.

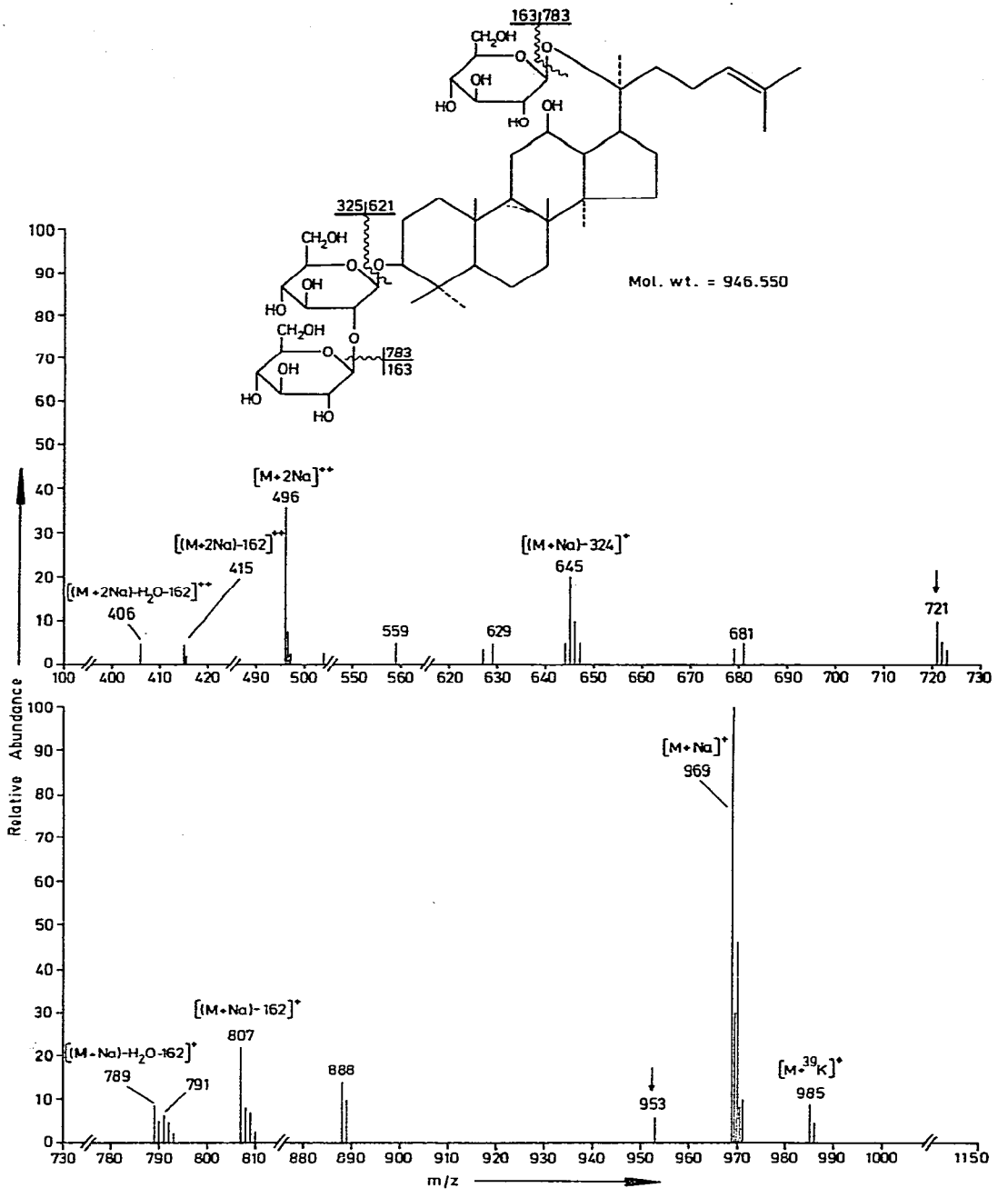

Fig. 7. FD mass spectrum of the HPLC fraction containing compound Rd. Eight mass spectra were averaged by the data system in a temperature range that corresponds to emitter heating currents between 30 and 40 mA. Whereas the noise level is comparable to that in Fig. 6, the base peak here represents 115,436 counts.

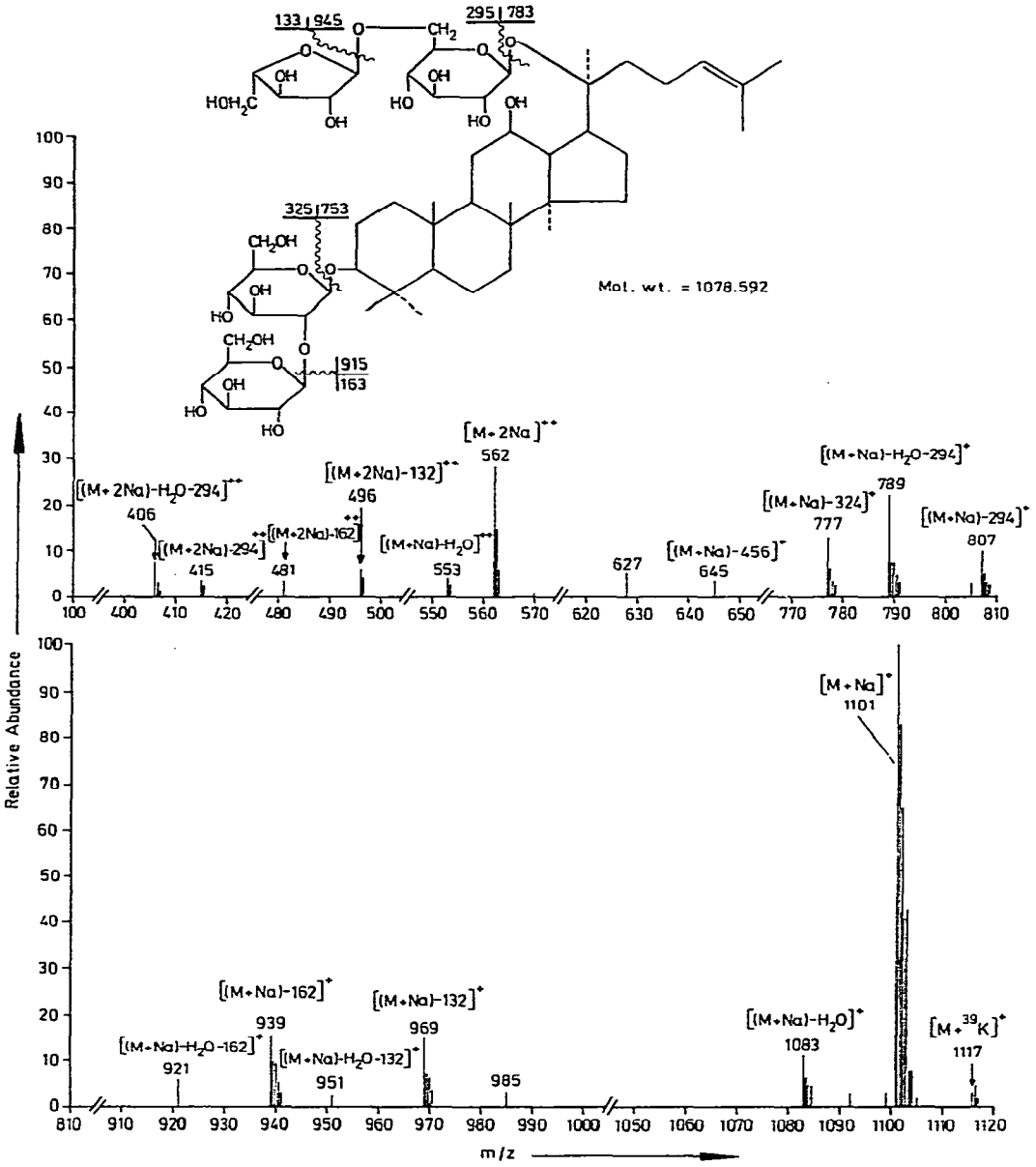

Fig. 8. FD mass spectrum of the HPLC fraction containing compound Rc. Within an emitter heating current in the range between 25 and 30 mA eight FD spectra were recorded and averaged. The base peak represents 57,776 counts; the background level (noise) is as in Figs. 6 and 7.

salt impurities, the $[M + {}^{39}K]^+$ ion at *m/z* 839 and its isotopic satellites are found. Loss of water from the molecule, probably from the aglycone, and cationization give the $[(M + Na) - H_2O]^+$ ion at *m/z* 805. An indication that the water loss is indeed from the aglycone and not the sugar moieties is derived from the signal at *m/z* 481, which can be explained as loss of water *after* cleavage of the disaccharide unit from

the glycosidic oxygen in position 6 of the aglycone. A general feature in FD-MS is the formation of doubly charged ions, and this is particularly valid for glycosides. This is clearly demonstrated by the FD ions at *m/z* 423, $[M + 2Na]^{2+}$, and *m/z* 431, $[M + Na + K]^{2+}$. The usefulness of these types of ion for additional confirmation of the molecular weight has been discussed previously in detail[21].

Cleavage of the terminal glucose from the molecule, proton transfer to the glycosidic oxygen and cationization are the processes that lead to the ion at *m/z* 661, explained as $[(M + Na) - 162]^+$. Similarly, the loss of both glucose units from position 6 of the aglycone occurs and, as the glycosidic oxygen is retained and proton transfer results in the hydroxyl function, an ion of comparable intensity is found at *m/z* 499 for $[(M + Na) - 324]^+$.

For compound Rd more fragmentation was found and, as shown in Fig. 7, the molecular weight of this ginsenoside is unambiguously determined by four series of FD ions. The $[M + {}^{39}K]^+$ ion at *m/z* 985, $[M + Na]^+$ at *m/z* 969, $[M + 2Na]^{2+}$ ion at *m/z* 496 and $[M + Na + {}^{39}K]^{2+}$ at *m/z* 504 all give information on the molecular weight. However, minor accompanying substances that could also be oligoglycosides cannot be excluded because the signals at *m/z* 953 and 721 are unlikely to be frag-

TABLE II

DETERMINATION OF THE MOLECULAR WEIGHTS AND ASSIGNMENT OF STRUCTURALLY SIGNIFICANT SIGNALS FOR CONFIRMATION OF THE SUGAR SEQUENCE IN THE HPLC FRACTION OF COMPOUND Rc BY FD-MS

*Type of ion**	*Relative abundance***	*Accurate mass*
$[M + K]^+$	+	1117.556
$[M + Na]^+$	+++++	1101.582
$[(M + Na) - X]^+$	+	1083.573
$[(M + K) - Z]^+$	+	985.514
$[(M + Na) - Z]^+$	+	969.540
$[(M + Na) - X - Z]^+$	+	951.529
$[(M + Na) - Y]^+$	+	939.529
$[(M + Na) - X - Y]^+$	+	921.519
$[(M + Na) - Z - Y]^+$	+	807.487
$[(M + Na) - X - Z - Y]^+$	++	789.427
$[(M + Na) - (Y - Y)]^+$	+	777.477
$[(M + Na) - X - (Y - Y)]^+$	+	759.466
$[(M + Na) - (Y - Y) - Z]^+$ $[(M + Na) - (Z - Y) - Y]^+$	+	645.434
$[(M + Na) - X - (Y - Y) - Z]^+$ $[(M + Na) - X - (Z - Y) - Y]^+$	+	627.424
$[M + 2Na]^{2+}$	++	562.286
$[(M + 2Na) - X]^{2+}$	+	553.281
$[(M + 2Na) - Z]^{2+}$	+	496.265
$[(M + 2Na) - Y]^{2+}$	+	481.266
$[(M + 2Na) - Y - Z]^{2+}$	+	415.238
$[(M + 2Na) - X - Y - Z]^{2+}$	+	406.233

* X = water; Y = glucose; Z = arabinose.

** The relative abundances for electrical detection are given with five levels: relative abundance 0–20% = +; 20–40% = ++; 40–60% = +++; 60–80% = ++++; 80–100% = +++++.

ments of Rd. A possible explanation is that in this compound the hydroxyl function in position C_{12} is labile and was exchanged against hydrogen (*e.g.*, after elimination of water). The molecular weight of this new compound would be 930 and the corresponding $[M + Na]^+$ ion at *m/z* 953 is detected with low relative abundance. Loss of one glucose unit $[(M + Na) - 162]^+$ would explain the ion at *m/z* 791 and the loss to two hexose sugars would give the ion at *m/z* 629. In view of the pronounced signals for elimination of water from the aglycone of Rd, *viz.*, $[(M + Na) - 162 - H_2O]^+$ at *m/z* 789, $[(M + Na) - 324 - H_2O]^+$ at *m/z* 627 and $[(M + 2Na) - 162 - H_2O]^{2+}$ at *m/z* 406, elimination of water from position 12 is a very likely process. Owing to the two possibilities for the loss of a terminal glucose from the molecule and the abundant $[(M + Na) - 162]^+$ ion, a doubly charged ion for the loss of a sugar unit *and* water was observed for the first time at *m/z* 406. Very interesting is a novel type of ion for $[(M + Na) - 162]^{2+}$, a signal which represents a doubly charged sequence ion.

The FD mass spectrum of Rc is shown in Fig. 8 and can be regarded as an optimal FD spectrum as far as structurally important fragmentation is concerned. As all types of ions described above for the other ginsenosides are detected, the accurate masses of these ions and their interpretation are listed in Table II in order to facilitate the understanding of the information obtained for molecular weight and structure.

ACKNOWLEDGEMENTS

This work was supported in part by the Deutsche Forschungsgemeinschaft, Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen and Umweltbundesamt. The authors thank Dr. D. P. May, Perkin-Elmer, Zürich, Switzerland, for technical assistance.

REFERENCES

1 F. Soldati and O. Sticher, *Planta Med.*, 39 (1980) 348.
2 O. Sticher and F. Soldati, *Planta Med.*, 36 (1979) 30.
3 R. Yost, J. Stoveken, A. Poile and W. MacLean, *Chromatogr. Newsl.*, 5 (1977) 28.
4 O. Sticher and F. Soldati, *Pharm. Acta Helv.*, 53 (1978) 46.
5 R. Yost, W. MacLean and J. Stoveken, *Chromatogr. Newsl.*, 4 (1976) 1.
6 *Leaflet 16/22/10/177 R*, Merck, Darmstadt, G.F.R., 1980.
7 E. Bombardelli, A. Bonati, B. Gabetta and E. Martinelli, *J. Chromatogr.*, 196 (1980) 121.
8 O. Sticher, F. Soldati, R. K. Joshi and D. Lehmann, *Fitoterapia*, 49 (1978) 147.
9 H.-R. Schulten, T. Komori and T. Kawasaki, *Tetrahedron*, 33 (1977) 2595.
10 H.-R. Schulten, T. Komori, T. Nohara, R. Higuchi and T. Kawasaki, *Tetrahedron*, 34 (1978) 1003.
11 H. Wagner, H. Habermeier, A. Liptak and H.-R. Schulten, *Planta Med.*, 37 (1979) 381.
12 K. Kudo, T. Nohara, T. Komori, T. Kawasaki and H.-R. Schulten, *Planta Med.*, 40 (1980) 250.
13 T. Komori, M. Kawamura, K. Miyahara, T. Kawasaki, O. Tanaka, S. Yahara and H.-R. Schulten, *Z. Naturforsch. C*, 34 (1979) 1094.
14 T. Komori, I. Maetani, T. Nohara, N. Okamura, T. Kawasaki and H.-R. Schulten, *Justus Liebigs Ann. Chem.*, in press.
15 H.-R. Schulten, *Z. Anal. Chem.*, 293 (1978) 273.
16 H.-R. Schulten and I. Stoeber, *Z. Anal. Chem.*, 293 (1978) 370.
17 I. Stoeber and H.-R. Schulten, *Sci. Total Environ.*, 16 (1980) 249.
18 D. A. Brent, P. de Miranda and H.-R. Schulten, *J. Pharm. Sci.*, 63 (1974) 1370.
19 H.-R. Schulten and D. Kuemmler, *Anal. Chim. Acta*, 113 (1980) 253.
20 U. Bahr, H.-R. Schulten, O. R. Hommes and C. Aerts, *Clin. Chim. Acta*, 103 (1980) 183.
21 H.-R. Schulten, *Int. J. Mass Spectrom. Ion Phys.*, 32 (1979) 97; and references cited therein.