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FIELD DESORPTION MASS SPECTROMETRY OF NATURAL PRODUCTS—III†

CORRINOID METAL COMPLEXES DERIVED FROM VITAMIN B_{12}

H. M. SCHIEBEL
Institute of Organic Chemistry, Technical University of Braunschweig, Schleinitzstrasse 2, D-3300 Braunschweig, Federal Republic of Germany

and

H.-R. SCHULTEN*
Institute of Physical Chemistry, University of Bonn, Wegelerstrasse 12, D-5300 Bonn, Federal Republic of Germany

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Abstract—Charged and uncharged metal complexes of secocorrin which are model compounds for the synthesis of biogenetically and pharmacologically interesting metal analogs of vitamin B_{12}, were investigated by field desorption mass spectrometry. The data contained in the spectra of these compounds permit an exact and reliable determination of the molecular weight. In addition structural information can be obtained based on typical fragmentation reactions. The support of the results received by high resolution and precise mass measurement was made by a direct analysis of the isotopic pattern of the corrinoid metal complexes. The first use of this combined method for field desorption mass spectrometry of a series of natural products of high molecular weight revealed that important indications can be obtained for the identification and characterisation of these compounds.

An attractive goal in corrin chemistry is the exchange of the central Co atom in vitamin B_{12} by other metals. The vitamin complexes which were modified in this way are important for the elucidation of the biogenesis and for the understanding of the biological function of vitamin B_{12} in higher organisms. Furthermore, as could recently be demonstrated,[1] these metal analogs have antimetabolic properties and are thereby of pharmacological interest as well. Until now all attempts to remove the Co atom and to substitute it by another metal were unsuccessful or led to a complete destruction of the B_{12}-ring system.[2] Corrin complexes with other central metal atoms than Co were therefore only accessible either by total synthesis[3,4] or by metalation of naturally occurring metal free vitamin B_{12} from phototrophic bacteria.[5] The high experimental effort of a total synthesis and the fact that only milligram amounts of suitable metal free corrins are available, was decisive for investigations, the goal of which is the removal of the Co by a reversible opening of the macrocyclus and its substitution by another metal. This would offer an alternative route for the synthesis of metal analogs of vitamin B_{12}. Very recently[6] in the case of the cobyrinic acid heptamethylester **1** which can easily be obtained by partial degradation of natural vitamin B_{12}, the regioselective and reversible opening of the ring system was successful. From the resulting open chain tetrapyrole the Co could be removed under formation of secocorrin **2** and substituted by other metals (Scheme 1).[7]

The analytical treatment of the expected preparative problems and of the following biochemical investigations requires a technique which permits a fast and reliable identification of the metal complexes. In this case the method of choice should be mass spectrometry. With the smallest amounts of substance, information on the molecular weight and to some extent the structure of the investigated compound can be obtained. In addition, information concerning the elemental composition of the individual ions by precise mass measurements and direct isotope analysis is possible.

In contrast to the metal complexes of the structurally related porphyrins, corrin metal complexes with the substitution pattern of vitamin B_{12} are unsuitable for the classical electron impact ionization mass spectrometry. The mass spectra of these compounds in no case contain the molecular ion of the analysed complexes. Further, they are rather complicated and only of little structural information because of simultaneously induced thermal *and* electron impact degradation processes. Additionally, as can be seen for vitamin B_{12}, as an example, various ionization techniques with less excitation energy such as chemical ionization, field ionization, and plasma desorption[8] do not produce intact molecular ions. Thermal sensitivity during evaporation is determining this behaviour not only for vitamin B_{12} but also for its derivatives.

With the method of field desorption mass spectrometry (FD-MS)[9] a separate evaporation of the substance, necessary for conventional ionization methods, is omitted. Ionization takes place from the solid phase of the adsorbed substance on the surface of the emitter. In contrast to electron impact ionization, field desorption of the investigated compounds provides simple and clear spectra with high relative intensities of the molecular ions. For cobyrinic acid heptamethylester **1**,[10] for derivatives of this ester[11] and for vitamin B_{12} itself[11] could recently be demonstrated that the method of field desorption enables

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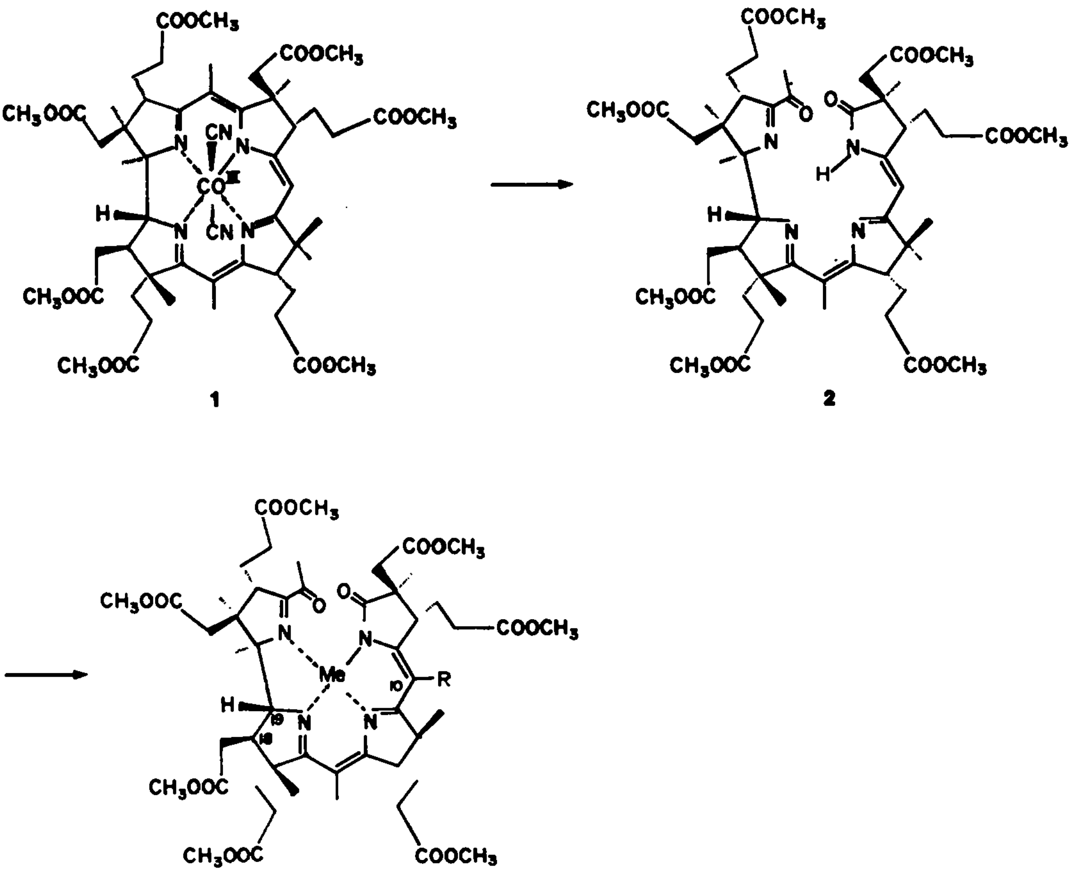
†Part II: H.-R. Schulten, T. Komori, T. Nohara, R. Higuchi and T. Kawasaki, *Tetrahedron* 32, 1003 (1978).



3: Me = PdC$_5$H$_7$O$_2$, R = H
4: Me = Ni$^+$CH$_3$COO$^-$, R = Br
5: Me = RhCl$_2$, R = H
6: Me = RhCl$_2$, R = Br
7: Me = RhBrCl, R = Br

Scheme 1. Chemical pathway for the synthesis of metal exchanged derivatives of vitamin B$_{12}$ and structures of the compounds investigated.

the mass spectrometric analysis of this class of compounds. Further a method for direct isotope analysis by FD-MS using spectra accumulation in a multichannel analyzer was introduced.[12]

In continuation of this work we now report on the application of high resolution FD-MS for the identification of charged and uncharged metal complexes of the open chain heptamethylester 2 whose central Co atom was substituted by other metals. For the first time the direct analysis of the isotopic pattern was used to support the high resolution data obtained.

RESULTS AND DISCUSSION

1. *Acetylacetonate complexes of secocorrin*

Generally, metal complexes of acetylacetonate do not have the characteristic features of salts but rather those of covalent compounds. This property determines, in addition, the behaviour of the Pd complex 3, whose axial position is occupied by the acetylacetonate anion. As shown in Fig. 1 the peaks of the intact molecular ions appear as radical cations at *m/e* 1213 to *m/e* 1218 corresponding to the Pd isotopes ^{105}Pd, ^{106}Pd, ^{108}Pd and ^{110}Pd. Using photographic detection the molecular ions for the metal complexes containing the isotopes ^{102}Pd (natural abundance 0.96%) and ^{104}Pd (natural abundance 10.97%) are not found. The base peak of the spectrum is the metal free protonated secocorrin 2 at *m/e* 1011.518. Its formation can be explained as a thermally induced elimination of the central Pd and its axial ligand before ionization. This process can also be observed in the case of electron impact ionization of simply substituted corrin metal complexes.[13] The temperature range between optimal desorption and starting thermal degradation is only small, accordingly it is critical to get the best anode temperature for desorption by manual approach. Therefore, using high resolution and photographic detection, for this compound only an FD-spectrum of low intensity and with an incomplete isotopic pattern of the molecular ion could be obtained. Electrical detection of the molecular ion resulted in a pattern, the intensities of which are only approximately in agreement with the calculated values. Because of the short desorption time of compound 3, it was not possible to accumulate more than 5 repetitive magnetic scans using a multichannel analyzer. For this investigation the average deviation of the experimental isotopic distribution for the molecular ions from the theoretical values was about 6%. As reported in previous FD investigations good agreement between the

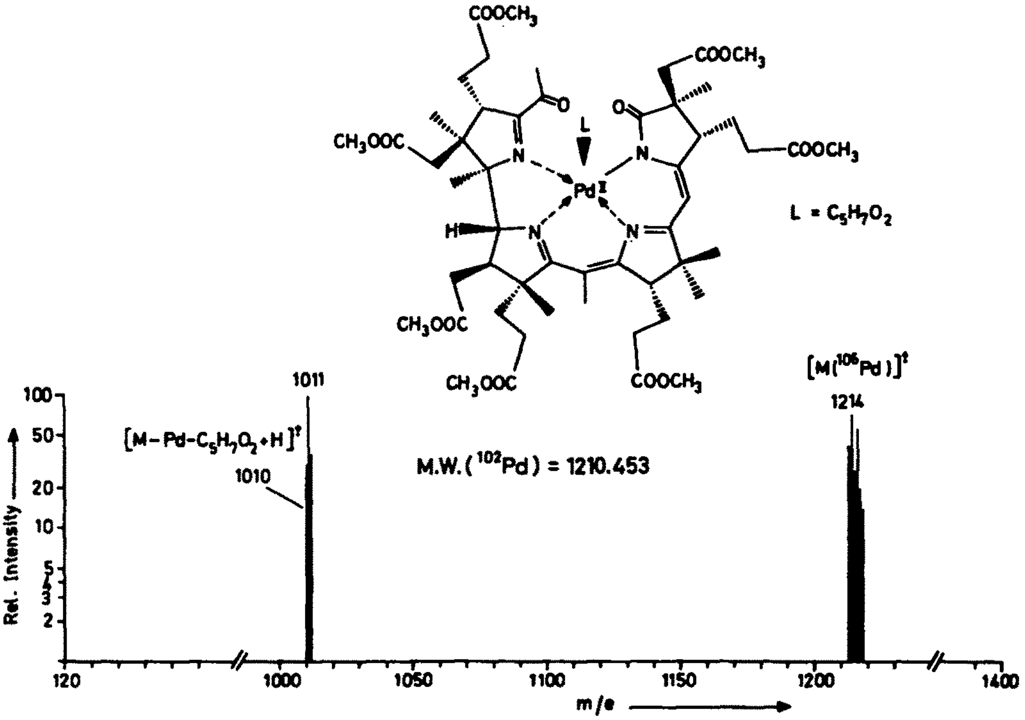


Fig. 1. Photographically recorded FD mass spectrum of compound 3. The exposure time of the photoplate was 12 min, the emitter heating current was raised from 0 to 70 mA during this time. The mass resolution obtained on the photoplate was 12.000 (at 50% valley definition).

theoretical and experimental isotopic pattern was achieved only in those cases where at least 30–50 magnetic scans could be recorded.[12] Thus it becomes clear that the length of the desorption time of the sample is an essential parameter for direct isotope analysis and is the limiting factor if this determination should be used to support the high resolution data of molecular ions or fragments.

However, in case the ion in question contains elements with a characteristic isotopic pattern (such as Pd) a good indication for the correct assignment of the elemental composition can be given even if only a few scans can be accumulated.

2. *Charged complexes of secocorrin*

Compound 4 represents a secocorrin metal complex with distinct salt character. The cation of the salt forms the peak group of highest mass and the base peak of the spectrum (Fig. 2). The high intensity of the cation of this compound is not only typical for charged secocorrin metal complexes but also, and in general, characteristic for the behavior of organic and inorganic salt cations under the condition of field desorption.[14] Only one fragment ion at *m/e* 1067.450 is observed. The formation of this fragment can be explained as a result of the substitution of the halogen in position 10 by hydrogen, an exchange which is possible by chemical reaction as well.[15] This substitution proceeds at the surface of the emitter and it is characteristic as well for corrins as for secocorrins with the substitution pattern of vitamin B_{12}. Besides the previously explained elimination of the central metal and its axial ligands, this reaction also represents a second type of thermally induced degradation which is characteristic for FD-MS for corrinoid metal complexes. The intensity of the fragments formed is strongly dependent on the temperature of the sample. Halogens are substituted more easily than the nitro group of the corresponding 10-nitro compound. In contrast to the acetylacetonate complex of the palladium 3, and on the basis of its ionic character, the nickel complex forms stable ion currents of high intensity during desorption. The longlasting, intense ion currents enable the analysis of the isotopic pattern in a simple way by spectra accumulation using a multichannel analyzer. Comparing the theoretical and experimental intensities of the molecular ion of compound 4 in Fig. 3 it is obvious that these intensities are in good agreement. The calculation of the error between experimental and theoretical values is shown in Table 1 and gives an average deviation of 0.4%.

Table 1. Direct isotope determination of the cation of compound 4

exp.	theor.	error
62.7	62.9	-0.2
30.7	38.7	+1.0
100	100	-
58.6	57.7	+0.9
44.7	48.2	-0.5
22.7	23.5	-0.8
12.2	12.3	-0.1
4.7	4.8	-0.1
2.4	2.3	+0.1
1.0	0.9	+0.1

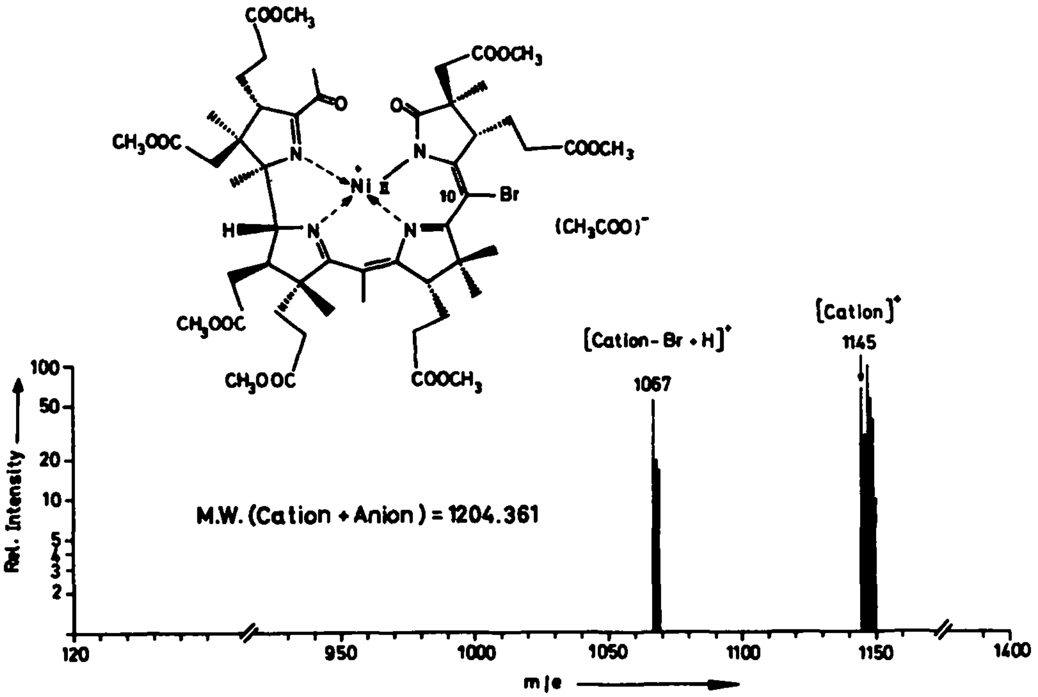


Fig. 2. Photographically recorded FD mass spectrum of compound 4. Exposure time 14 min, emitter heating current from 0 to 20 mA, the mass resolution for the adjustment of *m/e* 58 in the electrical detection mode was 10.000 (10% valley) and the mass resolution on the photoplate > 20.000.

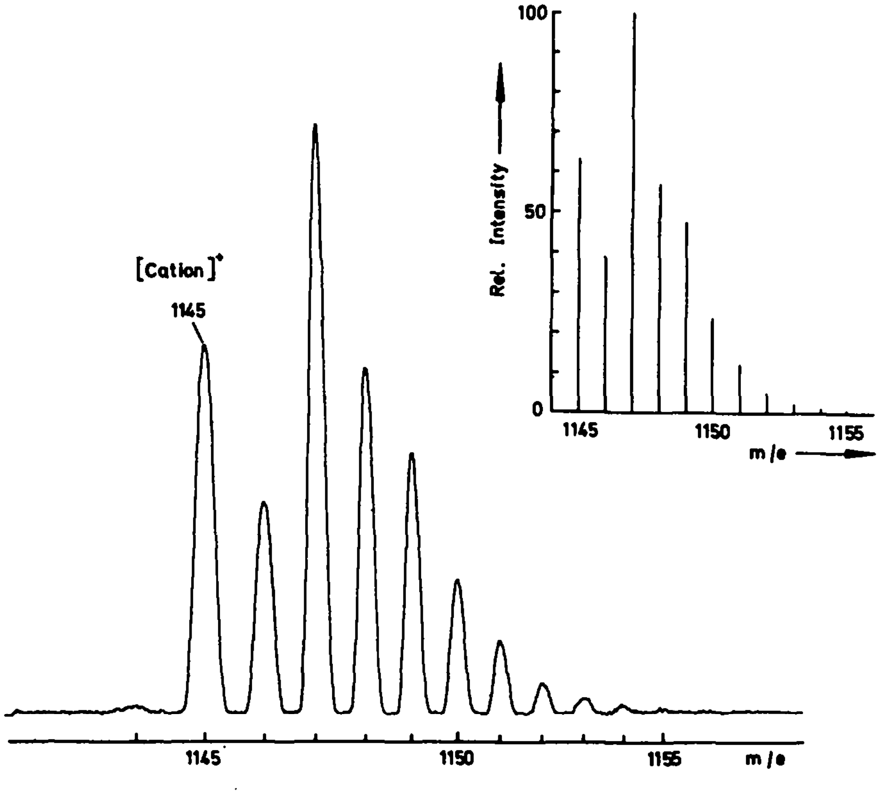


Fig. 3. Isotopic pattern of the cation of compound 4 at *m/e* 1145 obtained by spectra averaging using a multichannel analyzer (theoretical distribution in the inset). Experimental data: 35 scans, range 2^{11}, sweep time 5 sec. The average deviation of the experimental isotope distribution from the theoretical value (error) is 0.4%.

This drastically restricts the number of elemental combinations which are compatible with the precise mass measurements and thus supports the high resolution data in an excellent manner.

In order to demonstrate that the direct isotope determination in FD gives not only a good indication for the correct assignment of molecular ions or cations but also of FD fragments the plot of the [cation − Br + H]$^+$ ion is shown in Fig. 4. Using electric detection and the multichannel analyzer the found and expected values for the isotopic distribution of this fragment are in much better agreement than the data recorded with the photoplate (see Fig. 2).

3. *Uncharged complexes of secocorrin*

The rhodium compound 5 is an example for an uncharged secocorrin metal. Figure 5 displays the comparison of the photographically (Fig. 5a) and electrically (Fig. 5b) recorded FD-spectra of this metal complex. Again this comparison clearly visualizes the disadvantage of photographic detection. As in the case of the compounds 3 and 4, the isotopic pattern of the less intensive ions are incomplete because of the low dynamic range in the ion response of the photoplate. For example the pattern of the molecular ions of compound 5 at *m*/*e* 1182 to *m*/*e* 1187 is much better reflected by electric recording and averaging of 8 cyclic magnetic scans with the datasystem. The fragment of the highest mass at *m*/*e* 1147 represents the frequently observed loss of *one* axial ligand in the corrin system upon field desorption.[11] The peaks in the mass range 1110 indicate a more intense thermal strain during desorption. The fragment at *m*/*e* 1112 is formed by thermal elimination of *both* axial ligands and transition into a rhodium(I)-complex. This degradation process is similar to that of the Pd complex 3 and was first mentioned for the fragmentation behaviour of corrinoid metal complexes under electron impact conditions.[13] A further type of generally occurring thermally induced degradation reaction during FD is indicated by the fragments at *m*/*e* 1110 and 1111 and can be explained by a dehydrogenation of the secocorrin skeleton, presumably by formation of a Δ18, 19 double bond and elimination of both axial ligands. Depending on the experimental conditions and detectable by change of the fragment pattern, the resulting degradation products are recorded both as radical cation at *m*/*e* 1110 or as protonated ion at *m*/*e* 1111. This change of intensities in the course of desorption may also be observed directly by accumulation of these peaks using the multichannel analyzer.

The origin of the strong signal at *m*/*e* 1127, base peak in the spectrum which was recorded by photographic detection (Fig. 5a), is still unknown. The difference to the molecular ion and the isotopic pattern of this peak excludes it as a degradation product of the rhodium compound 5. Dynamic high resolution measurements using electric detection and accurate mass determinations on the photoplate revealed that this ion has a mass of *m*/*e* 1127.433 ± 2 mmu. The isotopic pattern is 100% (*m*/*e* 1127), 61.4% (*m*/*e* 1128), 21.9% (*m*/*e* 1129), 6.7% (*m*/*e* 1130) and 2.4% (*m*/*e* 1131). From these data it is clear that the unknown compound has a very similar elemental composition to the corrins investigated. For example a compound with the elemental composition $C_{53}H_{76}N_4O_{16}Rh$, theor. mass 1127.431 and an average deviation of the isotopic distribution of 0.8% is a possible solution. The presence of chlorine, bromine or a metal cation with more than 1 isotope in this accompanying substance can definitely be excluded. Careful chromatographic purifications of the original sample and repeated FD investigations revealed that the relative intensity of the ion at *m*/*e* 1127 is strongly reduced.

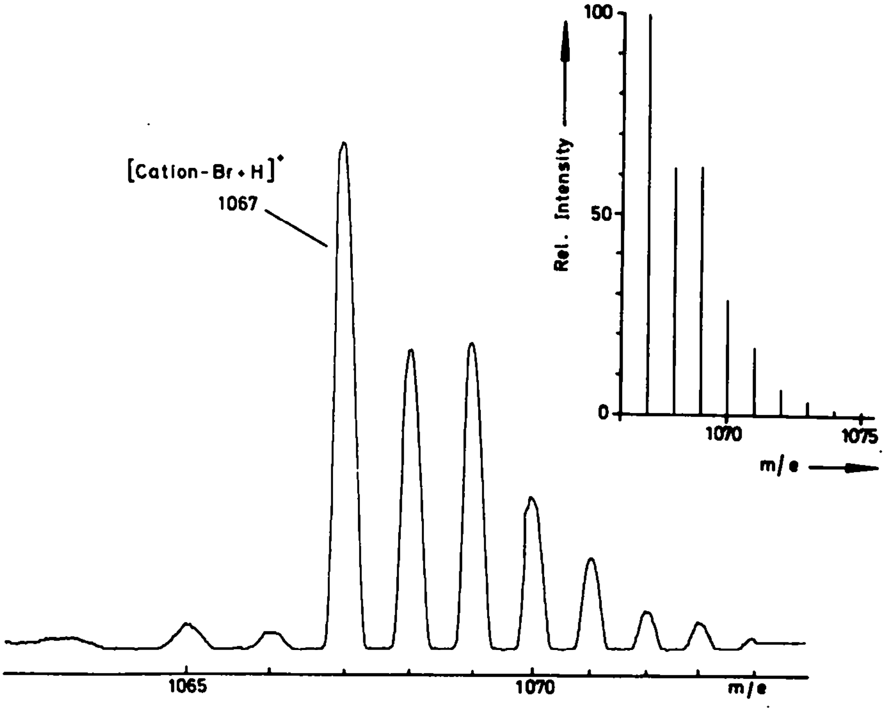


Fig. 4. Isotopic pattern of the [cation − Br + H]$^+$ fragment of compound 4 obtained by the technique described in Fig. 3 (theoretical distribution in the inset). Experimental data: 38 scans, range 2^{11}, sweep time 5 sec, error 1.3%.

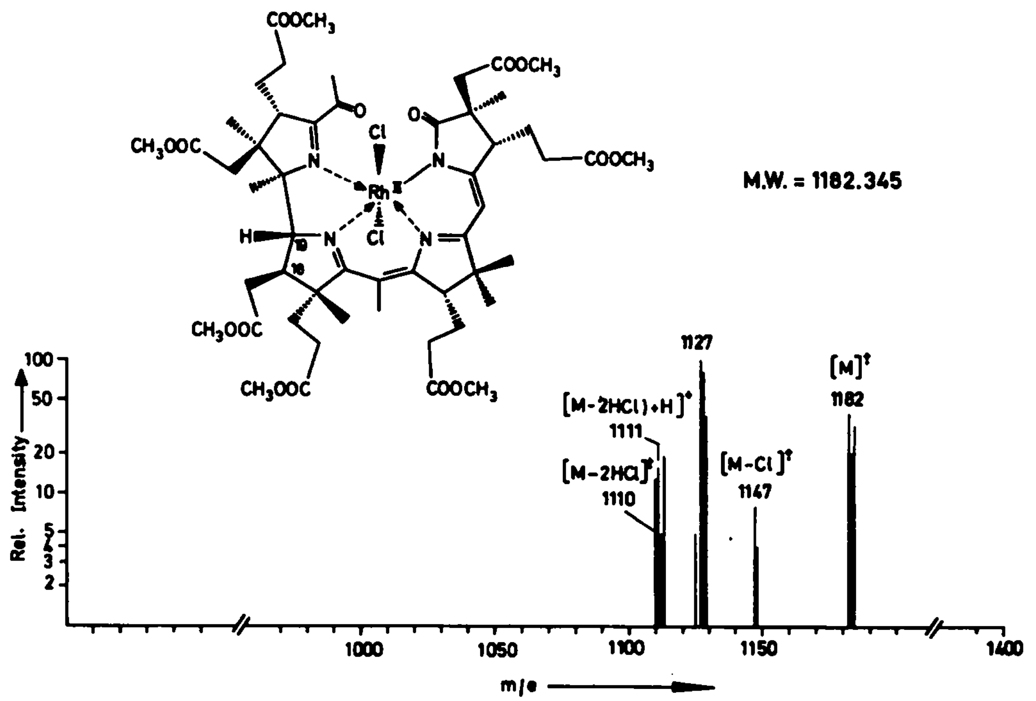


Fig. 5(a). Photographically recorded FD mass spectrum of compound 5. Exposure time 10 min, emitter heating current 20–50 mA, mass resolution as described in Fig. 2.

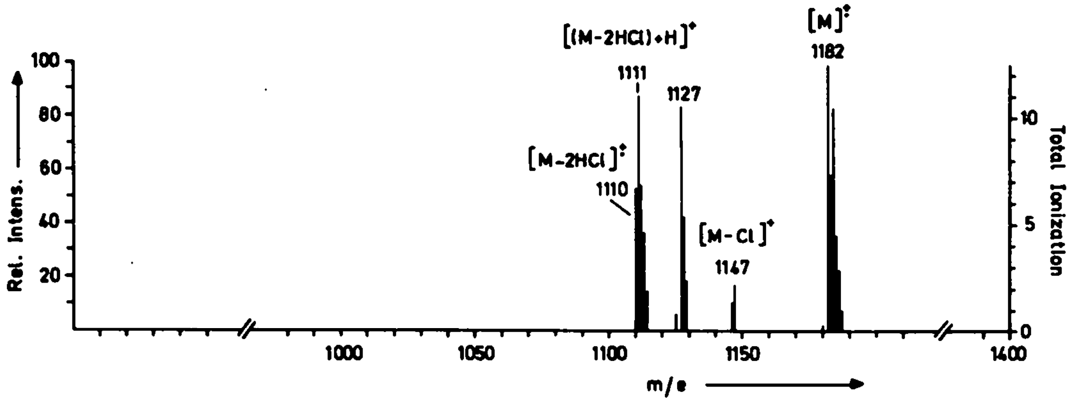


Fig. 5(b). Electrically recorded FD mass spectrum of compound 5. This spectrum shows the averaged intensities of the FD ions obtained in 8 repeated magnetic scans between 20 and 25 mA emitter heating current. Experimental data: scan speed 16 sec/decade according to 120 mu/sec, range of the datasystem (Varian MAT SS 200) 1 V, sampling frequency 6kHz, noise threshold 100 counts, count maximum 32760.

The spectrum of a sample which was obtained from secocorrin 4 after metalation and following bromination of position 10 with N-bromsuccinimide is displayed in Fig. 6. On account of the synthetic route, the dichloro complex 6 (M. W. 1260.256) was the expected compound. However, a second peak group, separated by 44 mass units, was surprisingly detected at *m/e* 1304. The observed mass difference and a knowledge of the general fragmentation behaviour of secocorrin metal complexes suggest a second compound with a similar substitution pattern. The analysis of the isotopic distribution and the high resolution data indicated that this compound was the rhodibyrinic acid complex 7 (M. W. 1304.205), formed by partial exchange of the axial ligand during reaction of dichloro-10-H-rhodibyrinic acid 5 with N-bromsuccinimide. The fragmentation of both complexes is strongly suppressed and limited to a few peaks of low intensity because of careful ionization at the best anode temperature of the investigated compounds. The origin of the fragments can be described by the typical degradation reactions previously mentioned, that is, loss of the axial ligands and substitution of bromine in position 10 by hydrogen. On account of the low intensities of the FD fragments, the deviations from the expected isotopic pattern are in this case particularly high. The experimental values of the isotopic pattern of the molecular ions are in rather good agreement with the values obtained by calculation (Fig. 7) in view of the fact that only *one* scan was recorded and analyzed. Simultaneously, and as in the case of the compound 5, this analysis makes possible a direct insight into the reaction during desorption. A slight increase of the emitter heat-

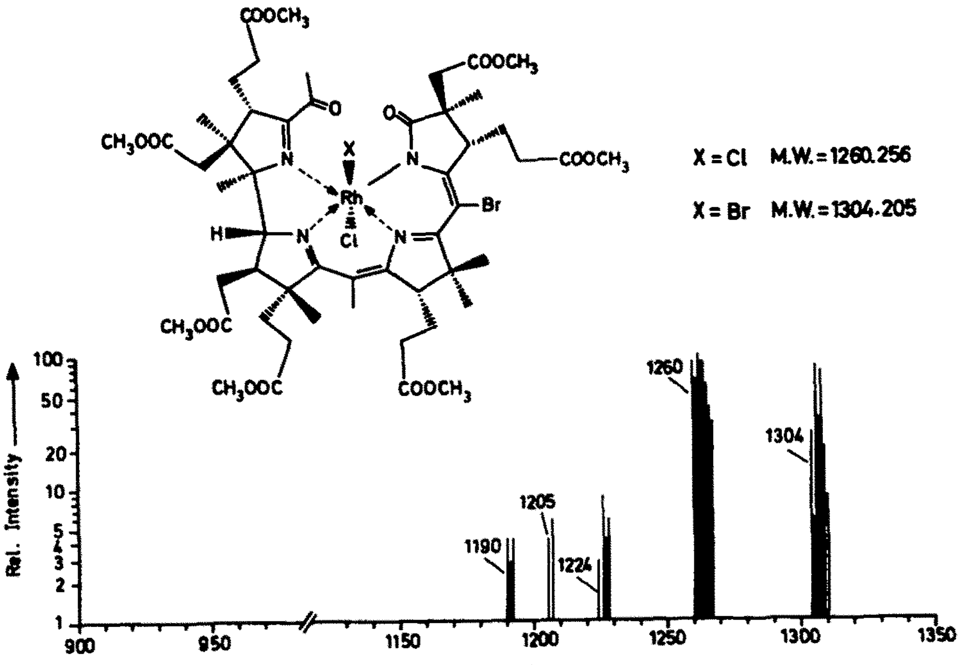


Fig. 6. Photographically recorded FD mass spectrum of compound 6 which contained compound 7 as an admixture. Exposure time 22 min, emitter heating current from 25 to 70 mA, mass resolution as described in Fig. 1.

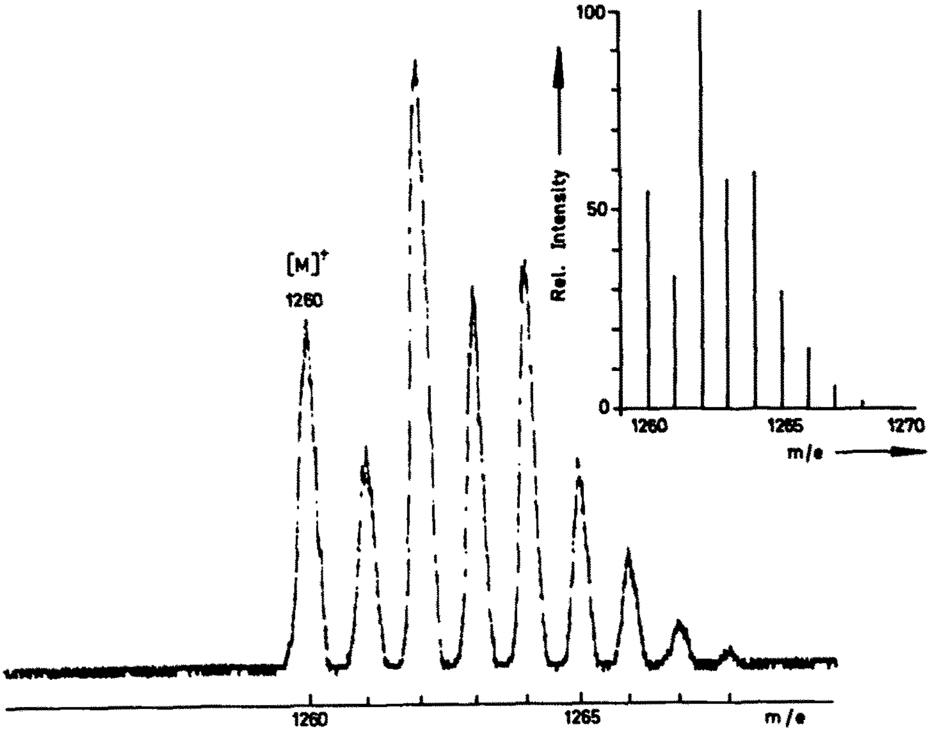


Fig. 7. Isotopic pattern of the molecular ion group of compound 6 obtained by storing a single magnetic scan at 28–30 mA emitter heating current in a multichannel analyzer, range 2^{11}, sweep time 5 sec. The average deviation of the isotopic distribution of the obtained signals from their theoretical value is about 6%. It should be mentioned, that at higher emitter heating currents this deviation is rapidly increased. At 30–35 mA for example, compound 6 gives an error of about 10%. It is assumed, that mainly field induced surface processes on the emitter surface contribute to these larger errors since intense $[M-H]^+$ and $[M-2H]^+$ ions are found at the higher temperatures of the emitter.

ing current leads to an increasing protonation of the molecular ion. This process is superimposed by the dehydrogenation of the corrin system starting at higher sample temperatures and it results in an appreciable shift of the original isotopic pattern.

SUMMARY

High molecular ion intensities are the general feature of the FD investigations of thermally labile metal complexes. Under the condition of field desorption and dependent on the temperature of the sample, four characteristic types of thermally induced degradation reactions can be observed for secocorrin metal complexes with the substitution pattern of vitamin B_{12}.

1. Demetalation.
2. Exchange of the substituent in position 10 by hydrogen.
3. Loss of the axial ligands and transition in a metal (I)-complex.
4. Dehydrogenation of the corrin skeleton.

The FD-spectra of these compounds thus contain not only precise data concerning the molecular weight and the central metal atom of the complex but also information concerning the structure of the investigated secocorrin complex by variation of experimental conditions. The method of direct isotopic analyses by spectra accumulation using a multichannel analyzer supplies useful information that supports the high resolution data. The correct assignment of the molecular weight is confirmed and the interpretation of the fragments is facilitated. Moreover, for organo-metallic compounds (as here for the corrins) the central metal cations are clearly indicated, in particular, if more than one metal isotope is involved.

EXPERIMENTAL

The FD-spectra were produced on a commercial Varian MAT 731 instrument with a combined EI/FI/FD ion source. The high resolution spectra were recorded by photographic detection with vacuum evaporated AgBr photoplates (Ionomet, Waban, Mass., U.S.A.). Desorption of the sample was regulated at a reading of the total ion current monitor of 2–3 V. This corresponds to an FD ion current which impinges on the entrance slit of 2×10^{-15} A. The mass resolution obtained is given in the figure captions. For nominal mass measurements reference masses were taken from the EI mass spectra of tris(perfluoroheptyl)-S-triazine. For accurate mass measurements on the photoplate the use of FD signals of a reference compound gives an average accuracy in the mass determination of about 1 ppm using a Gaertner-M-1205 PC Comparator. The electrically recorded low and high resolution spectra (in the text the theoretical masses are given) were obtained using the Varian MAT datasystem SS 200. For the analysis of the isotopic pattern a multichannel analyzer of the type Varian C-1024 cat was triggered by the magnetic scan of the mass spectrometer.[12] The theoretical values for the isotopic pattern of the corrinoids were calculated with a locally developed BASIC-program using the SS 200 datasystem. Our program enables the use of 36 nuclids and incorporates recently published isotope distributions.[16] Field desorption emitters, used in all experiments, were prepared by high temperature activation of 10 μm diam. tungsten wires.[17] The distribution and morphology of the microneedles produced were as shown previously.[18] FD emitters with an average length of 40–60 μm for the carbon microneedles were used as standards. Field desorption was performed using direct heating of the FD emitter wire. The corresponding emitter heating currents are given in the figure captions. The ionization efficiency and the adjustment of the FD emitter were determined by means of m/e 58 of acetone in the field ionization mode. All FD spectra were produced at an ion source pressure of 5×10^{-6} Torr and ion source temperatures 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. CH_2Cl_2 was used as solvent for all compounds. In general, approximately 1×10^{-6} g was applied as sample to the standard emitter via the syringe technique.[18]

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