152. Electrospray-Ionization Mass Spectrometry: Detection of a Radical Cation Present in Solution: New Results on the Chemistry of (Tetrahydropteridinone)-Metal Complexes[1])

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Dedicated to Prof. Dr. Dr. *Wolfgang Pfleiderer* on the occasion of his 65th birthday

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This paper marks the first reported detection of radical cations by Electrospray-Ionization Mass Spectrometry (ESI-MS). Electron Spin Resonance (ESR) measurements have proven that the detected radical cation existed already in solution and has not been generated by the electrospray ionization technique. However, we observed that the radical cation can be generated by changes in the ionization conditions. A molar mixture of 2-amino-5,6,7,8-tetrahydro-5-methylpterin-4(4*H*)-one dihydrochloride (= 5,6,7,8-tetrahydro-*N*(5)-methylpterin·2 HCl, *N*(5)-MTHP·2 HCl), and tris(pentane-2,4-dionato)iron(III) in MeCN at pH 2–3 leads to the formation of a [bis(pentane-2,4-dionato)(2-amino-5,6,7,8-tetrahydro-5-methylpteridin-4(4*H*)-one)]iron complex (= [bis(pentane-2,4-dionato)(5,6,7,8-tetrahydro-*N*(5)-methylpteridin)]iron complex) which can be detected by ESI-MS. The results suggest that this complex might be an Fe^{II} radical cation, which could possibly be a suitable model complex for the active center of the phenylalanine hydroxylase. In the same solution, the stable radical cation of *N*(5)-MTHP is identified by ESI-MS and ESR.

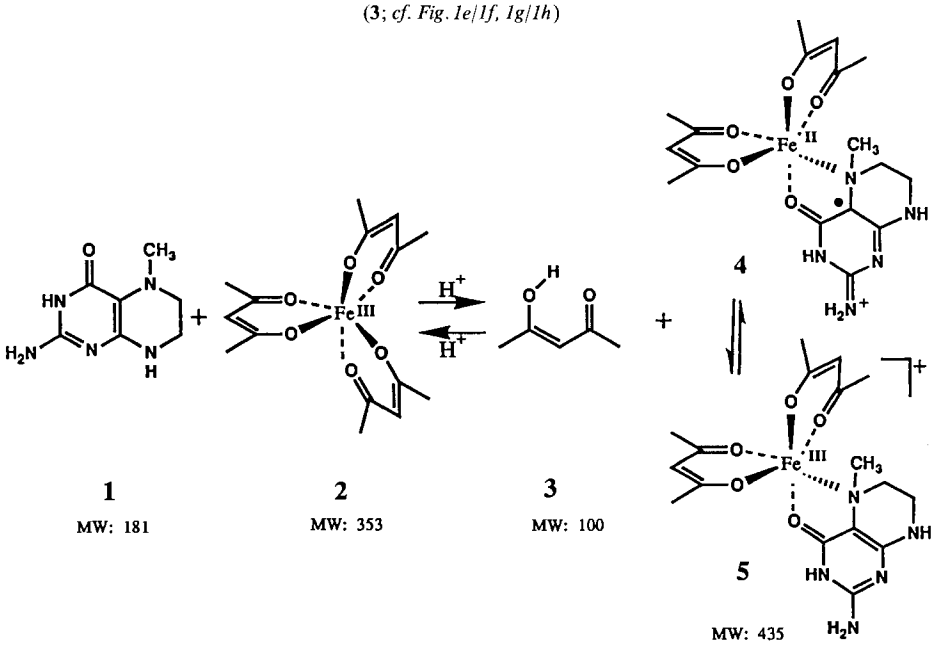
Introduction. – The coupling of Electrospray Ionization (ESI) with mass spectrometers started in the 1980s [2] [3]. Since that time, there has been a rapid advance in the instrumentation and application of ESI-MS [2] [3]. ESI-MS provides new means to analyze non-volatile, polar, and thermally labile compounds. It has gained recent attention because of the ability to produce multiply charged ions from large biomolecules such as proteins and oligo nucleotides [4] [5] making them amenable to analysis by most mass spectrometers. However, ESI-MS is equally well suited for compounds of low molecular weight, which are difficult to ionize intact by other methods, for example small peptides [3] [6], sulfonated azo dyes and steroids [6], hydroxylated polyamine derivatives [7], marine toxins [8], organoarsenic species [9], metalloporphyrines [10], and transition-metal complexes [1] [11].

[1]) Part 94 of the series 'Pterin Chemistry'. Presented at the '6th International Conference on Pteridines and Related Biogenic Amines and Folates', Seoul (South Corea), June 7–10, 1992. Part 93: [1].

We realized that ESI-MS can even be used to detect radical cations that exist in solution, whereas *Van Berkel et al.* [10] [12] reported the observation of radical cations formed during the ESI process.

It is known that (1′*R*,2′*S*,6*R*)-2-amino-6-(1′,2′-dihydroxypropyl)-5,6,7,8-tetrahydropterin-4(3*H*)-on[2] (= 6*β*-5,6,7,8-tetrahydro-L-biopterin[3]) is a cofactor of several monooxygenase enzymes. Best known is the hydroxylation of phenylalanine to tyrosine by the enzyme phenylalanine hydroxylase. More than twenty years ago, it was already assumed that during the enzymatic reaction the 6*β*-5,6,7,8-tetrahydro-L-biopterin[3]) is directly coordinated to Fe^{III} and Fe^{II} [1] [14]. We can demonstrate that tris(2,4-pentandionato)iron(III) (**2**), usually called acetylacetonate iron and abbreviated as [Fe^{III}(acac)$_3$], reacts with 2-amino-5,6,7,8-tetrahydro-5-methylpteridin-4(4*H*)-one[2]) (**1**) (= *N*(5)-methyltetrahydropterin, *N*(5)-MTHP[3])) to give a Fe complex (**4**/**5**; *Scheme 1*), which is reasonable model complex for the investigation of the hydroxylation reaction of phenylalanine. We can assume the proposed metallorganic complex **4** to be a radical cation and we can prove the formation of the radical cation **1′** of *N*(5)-MTHP (*Scheme 2*), which was proposed 25 years ago [1] [14].

Scheme 1. *The Reaction of* **1** · 2 HCl *and* **2** *at pH 2–3, Yielding the Fe Complex* **4**/**5** *and Pentane-2,4-dione* (**3**; *cf. Fig. 1e/1f, 1g/1h*)



Results and Discussion. – A 10^{-3} M solution of **1** · 2 HCl in MeCN containing 10% H_2O (pH 2–3) shows the $[M + H]^+$ signal in the ESI-MS at *m*/*z* 182 as well as protonated clusters of **1** (*Fig. 1, c*). The formation of the clusters of **1** at *m*/*z* 363 $[2M + 2H]^+$, *m*/*z* 453 $[5M + 2H]^+$, *etc.* are most likely due to the high concentration of compound **1** in the

[2]) Name according to the *IUPAC* rules.

[3]) Name according to the designation rules proposed in [13] for pterin derivatives.

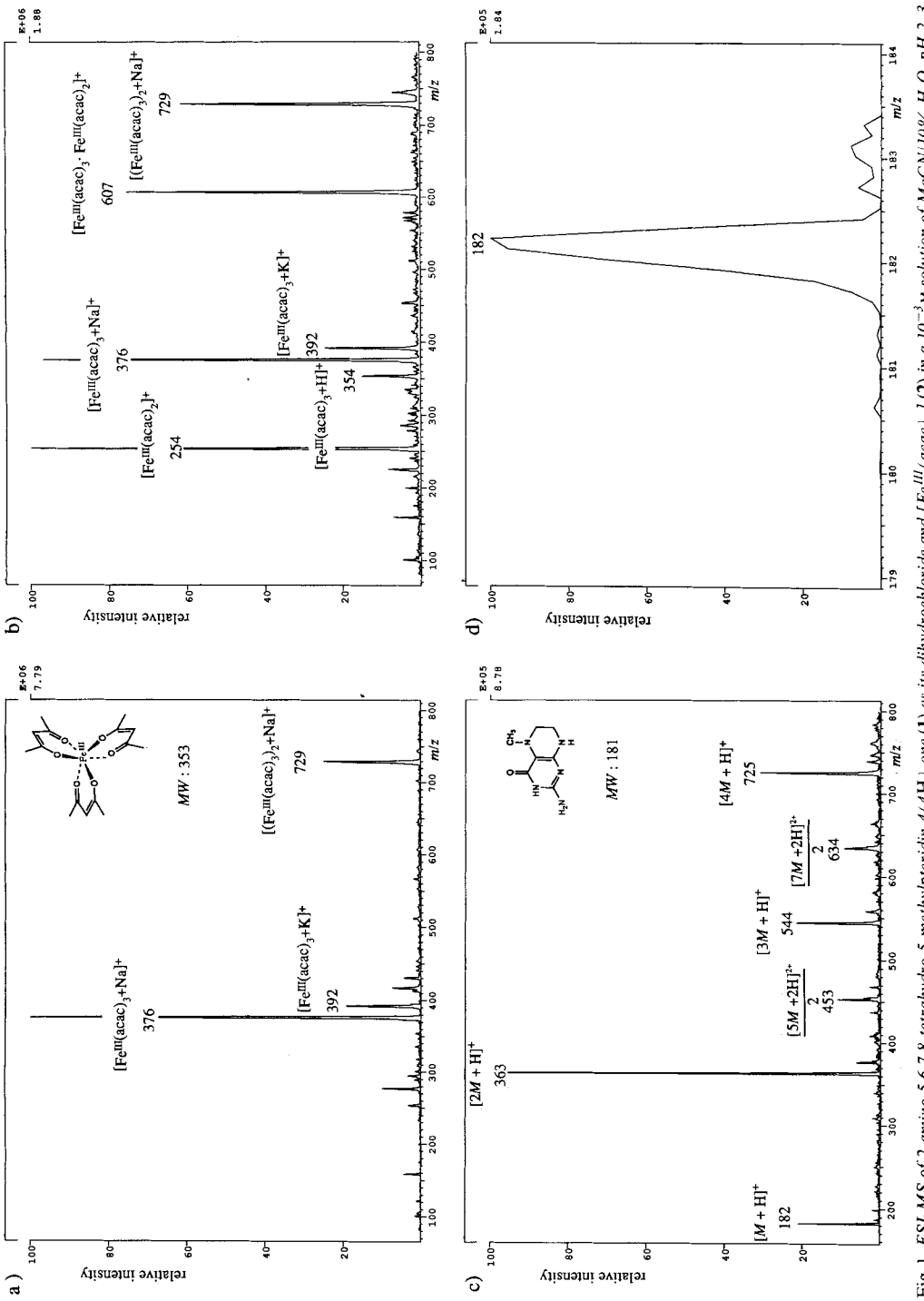


Fig. 1. *ESI-MS of 2-amino-5,6,7,8-tetrahydro-5-methylpteridin-4(4H)-one* (**1**) *as its dihydrochloride and [Fe^{III}(acac)$_3$]* (**2**) *in a 10^{-3}* M *solution of MeCN/10% H_2O, pH 2–3* (except for *Fig. 1a*). Ionization conditions: tube lens −34 V; lens 1.1 −26 V. *a*) [Fe^{III}(acac)$_3$] (**2**) at pH 2–3. *b*) [Fe^{III}(acac)$_3$] (**2**) at pH 7. *c*) 2-Amino-5,6,7,8-tetrahydro-5-methylpteridin-4(4*H*)-one as its dihydrochloride (**1** · 2 HCl). *d*) Enlargement of the sector of the [*M* + 1]$^+$ signal of compound **1**.

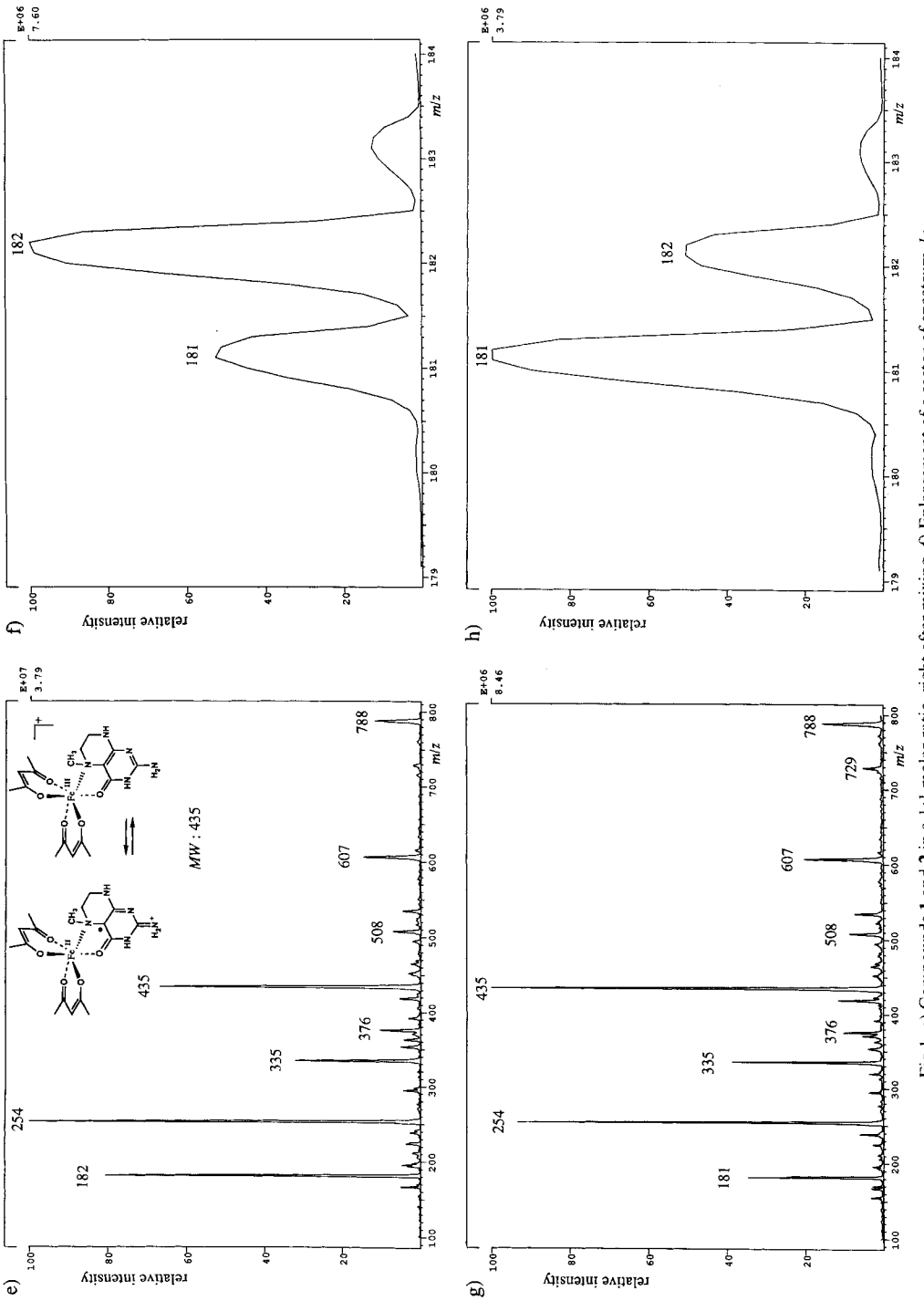
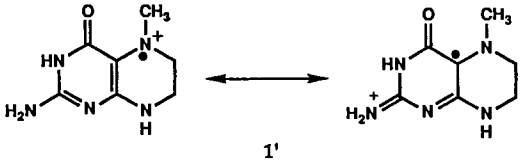


Fig. 1. *e*) Compounds **1** and **2** in a 1:1 molar ratio, right after mixing. *f*) Enlargement of a sector of spectrum *1e*. *g*) Compounds **1** and **2** in a 1:1 molar ratio, 20 min after mixing. *h*) Enlargement of a sector of spectrum *1g*.

solution [15]. Spectra of pure $[Fe^{III}(acac)_3]$ (**2**; 10^{-3} M) are given in *Fig. 1, a, b* at different pH values.

The proposed reaction of compound **1** and **2** is given in *Scheme 1*. In the ESI-MS of the 1:1 molar mixture **1**/**2** at pH 2–3 (*Fig. 1, e*) several new signals appear, which are not present in the spectra of the pure compounds. The most intensive new signals are at *m/z* 435, 335, and 181. The signal at *m/z* 254 which corresponds to $[Fe^{III}(acac)_2]^+$ increases drastically. Ion *m/z* 181 corresponds to the radical cation **1′** of compound **1** (*Scheme 2*), which is generated through the dissociation of complex **4** in solution. The cluster ions of compound **2** at *e.g. m/z* 607 and *m/z* 729 are still present, whereas those of compound **1** are missing. The signal observed at *m/z* 435 corresponds to our compound of interest **4**/**5** (*Scheme 1*). The solution that gave the spectra *Fig. 1, e, g* kept under Ar still showed the signal at *m/z* 435 after more than three weeks.

Scheme 2. *The 2-Amino-5,6,7,8-tetrahydro-5-methylpteridin-4(4*H*)-one Radical Cation* **1′**

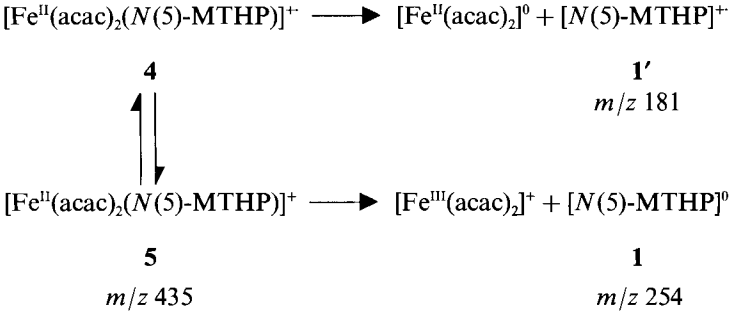


1′

Arguing from analogy to the known 6β-1,5-quinoid-7,8-dihydro-6*H*-L-biopterin complex[3]) [16] and the detected molecular weight at *m/z* 435, one can propose **4** to be a radical cation present in solution. Ion with the signal at *m/z* 335 is best explained by the loss of one neutral pentane-2,4-dione (**3**) from compound **4**. An increase of *m/z* 254 can be marked, representing $[Fe^{III}(acac)_2]^+$ or polymers of this compound, which would lead to the same signal [17].

Comparing *Figs. 1, e, f* and *1, g, h*, one can observe that the ESI-MS taken 20 min later (*Fig. 1, g, h*) shows an increased intensity of the signal at *m/z* 435, and the intensities of *m/z* 182 and 181 are in inverse proportions. As expected the formation of complex **4**/**5** goes along with a decrease of compound **1**, although the signal of **1** never disappears.

One can picture two possibilities for the dissociation of complex **4**/**5** in solution.



In the first case, the pteridinone ligand has given one electron to the Fe-center of the complex to form the Fe^{II} species **4** and leaves as a mesomerically stabilized radical cation

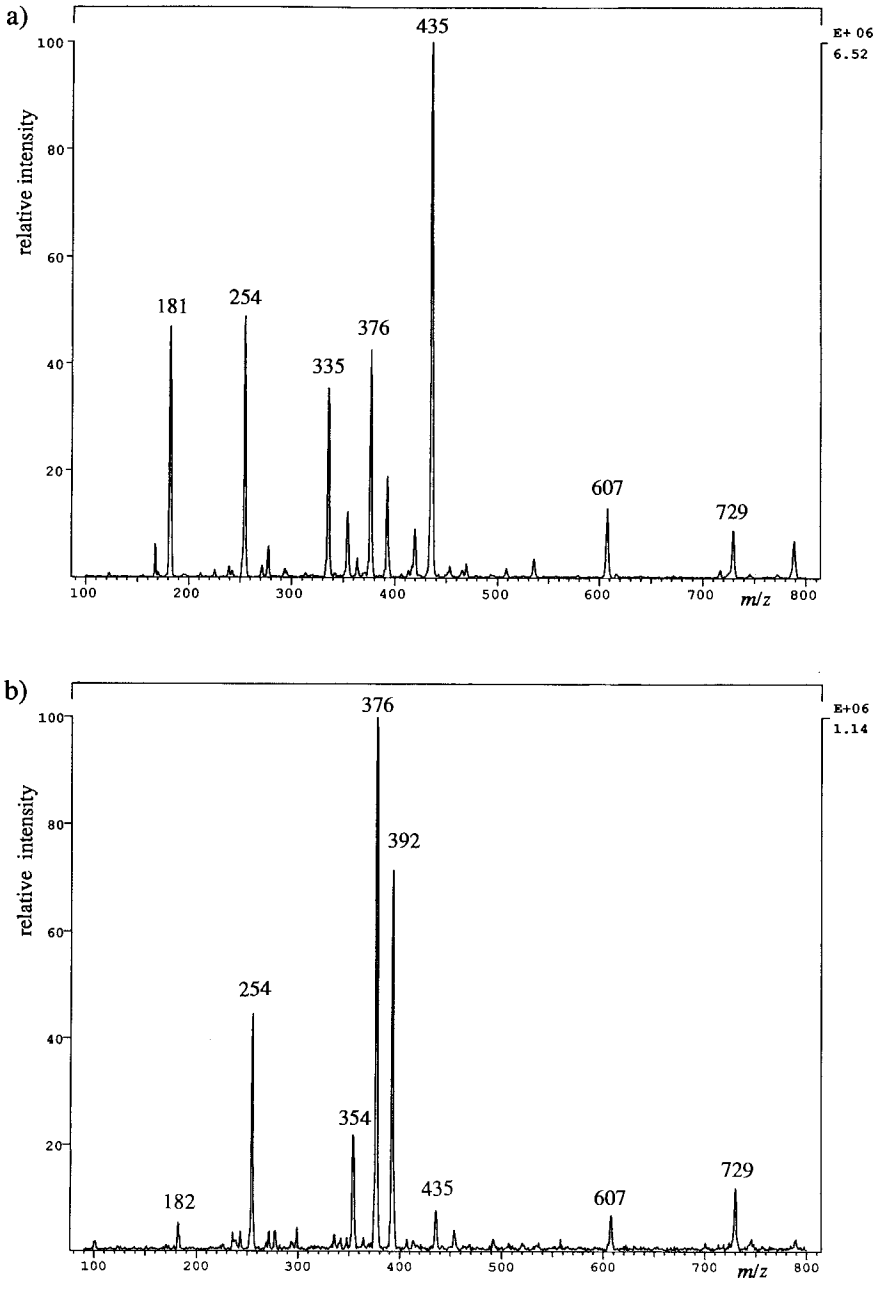


Fig. 2. a) *ESI-MS of a 1:1 molar solution of compounds* **1** *and* **2** (*cf. Fig. 1, g*).
b) *Solution as in* Fig. 2, a *with an additional excess of pentane-2,4-dione* (**3**)

1′, giving a signal at *m/z* 181. Electrospray ionization is an atmospheric-pressure-ionization method; this allows one to assume that the $[Fe^{II}(acac)_2]^0$ species is instantly oxidized to $[Fe^{III}(acac)_2]^+$ that leads to a signal at *m/z* 254. Looking at the redox potentials and cyclovoltammeric data of similar $Fe^{II/III}$ complexes this is very likely to happen [17] [18]. Another reason, why we do not observe the protonated $[Fe^{II}(acac)_2 + H]^+$ species is its low basicity due to which it is not readily protonated, but it is known that it tends to form polymers in solution [17], and we observe $[(Fe^{II}(acac)_2)\cdot(Fe^{II}(aca)_2)]^+$ at *m/z* 508 (*Fig. 1, e, g*), a signal that is not present in the pure $[Fe^{III}(acac)_3]$ (*Fig. 1, a, b*).

The second possibility is that we observe the dissociation of $[Fe^{III}(acac)_2(N(5)\text{-MTHP})]^+$ (**5**), which leads directly to $[Fe^{III}(acac)_2]^+$, giving the signal at *m/z* 254, and the neutral compound **1** which will be protonated and give the signal at *m/z* 182. This explains why the signal at *m/z* 182 never disappears, but seems to be regenerated. Another possibility for the regeneration of $[M + H]^+$ at *m/z* 182 is the following reaction, proposed by *Van Berkel et al.* [12]:

$$2\,[N(5)\text{-MTHP}]^{+\cdot} + H_2O \longrightarrow 2\,[N(5)\text{-MTHP} + H]^+ + \tfrac{1}{2}\,O_2$$

Contrary to what we would expect from this reaction, we can prove complex **4**/**5** to be stable for more than three weeks, whereas, in the presence of O_2, the signal at *m/z* 435 disappears within a short time[4]).

Further proof that *Scheme 1* shows an equilibrium was attained by the following experiment: to the 1:1 molar solution of compound **1** + **2** (*Fig. 2, a*; *cf. Fig. 1, g*), a large excess of pentane-2,4-dione (**3**) was added. *Fig. 2, a, b* demonstrates that an excess of compound **3** shifted the equilibrium indicated in *Scheme 1* to the left side. The spectrum in *Fig. 2, b* shows besides compound **1** dominantly signals of compound **2**, and only a minor signal of compound **4**/**5** is detectable.

The confirmation that the signal at *m/z* 181 is due to the radical cation **1′** was deduced from an ESR measurement (*Fig. 3*). Even after 20 h, a 10^{-6} M solution of **1** + **2** in a 1:1 molar ratio in $MeCN/H_2O$ (pH 2–3) showed the signal of the cationic radical. The

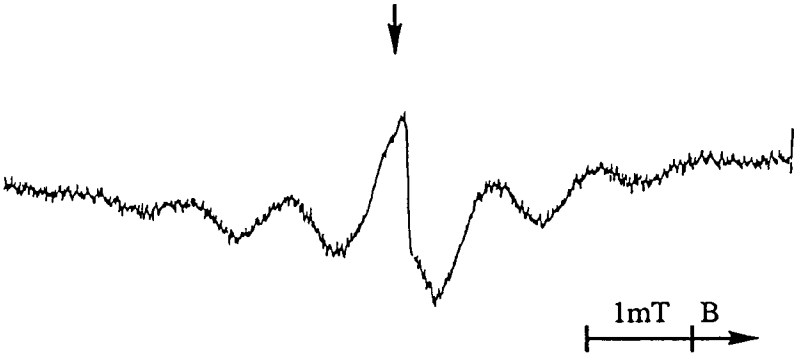


Fig. 3. *ESR Spectrum of the 1:1 molar mixture of* **1** *and* **2** *in* $MeCN/H_2O$, 10^{-6} M, *pH 2–3, after 20 h*

[4]) Systematic investigations concerning the O_2 uptake will follow.

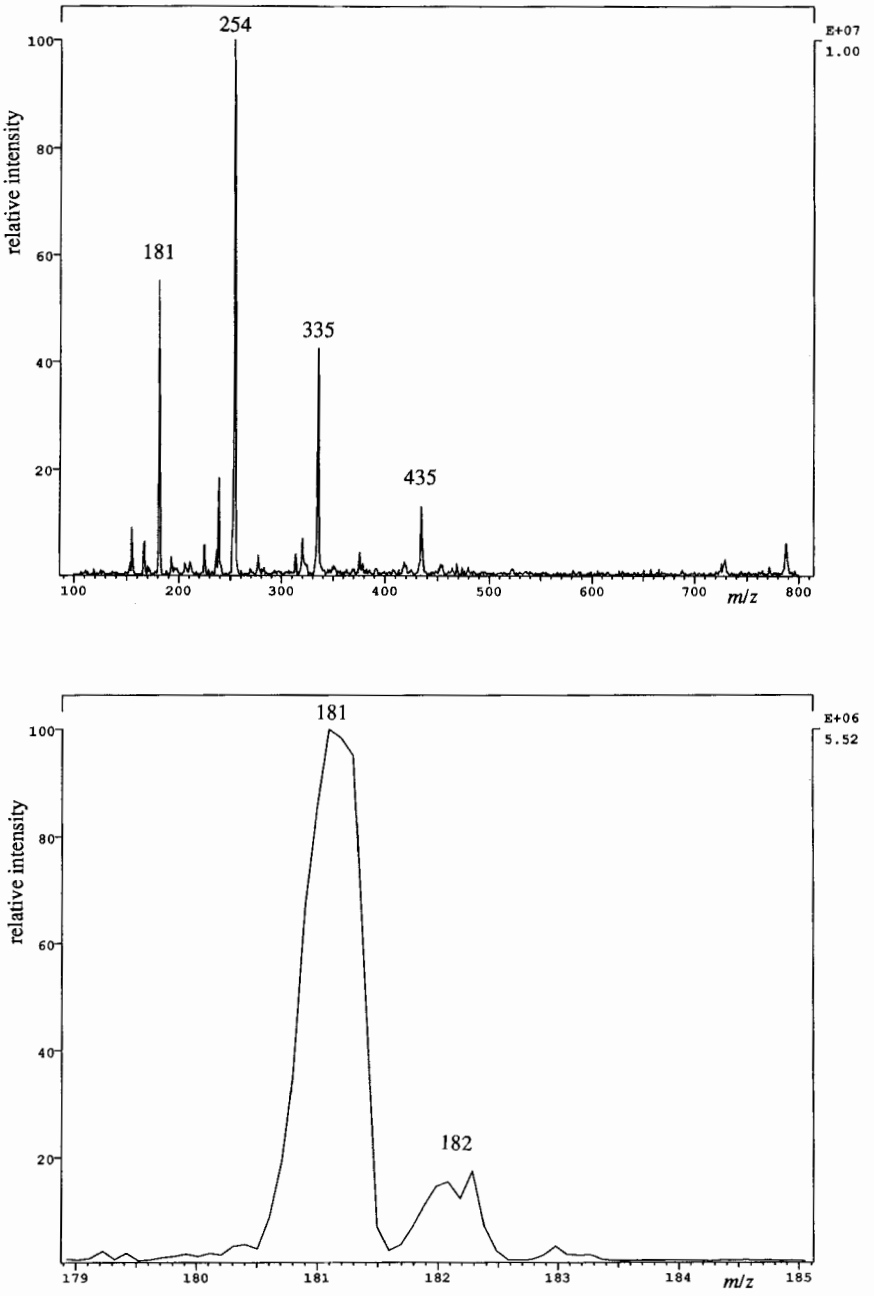


Fig. 4. *ESI-MS of the solution giving the spectra shown in* Fig. 1, g, h, *but the tube lens now is set to +85 V.* All other tune parameters remain the same.

spectrum (*Fig. 3*) exhibits seven equally spaced lines (g = 2,003; a = 1 mT) with approximate intensity ratios of 1:5:11:14:11:5:1, corresponding to the known ESR spectra of tetrahydropteridinone radicals formed by acidic H_2O_2 oxidation [14] [19]. Unfortunately, the spectrum in *Fig. 3* is superimposed by a paramagnetic impurity in the ESR cavity (marked by arrow) which was also present in the blank experiment (solution without **1** and **2**).

Complex **4** could not be identified by ESR spectroscopy. Possibly, strong anisotropic interaction between the Fe^{II} center and the unpaired electron of the ligand leads to extremely fast spin relaxation and, therefore, broadening of the ESR signal of complex **4**.

Van Berkel et al. showed that radical cations can be formed by ESI-MS [10] [12]. We observed that it is also possible to generate the radical cation **1′** by dissociation of the radical-cation complex **4** during the ionization process by changing the voltage at the tube lens (*Fig. 4*). When the voltage at the tube lens is more positive, the intensity of the peak at *m/z* 435 (**4**) drops drastically, whereas the intensity of the signal at *m/z* 181 increases. The relative intensities change stepwise according to the changes of the voltage at this lens. *Fig. 1, g, h* shows the spectrum at standard conditions (tube lens −34 V); the spectra depicted in *Fig. 4* are recorded with the tube lens at +85 V. This observation confirms that the radical cation **1′** is formed through dissociation of complex **4**. However, we want to stress that the ESR measurement proves that the radical cation of **1** already existed in solution, and that *Figs. 1a–h* give a picture of the situation in solution.

Experimental Part

The ESI mass spectra were recorded on a *Finnigan TSQ 700* with an electrospray ion source. For standard ionization conditions, see *Fig. 1*. All changes are marked, *e.g. Fig. 4*. The ESR spectrum was run on a *Bruker ESP 300*.

2-Amino-5,6,7,8-tetrahydro-5-methylpteridin-4(4H)-one (**1**) was synthesized by a small modification of *Matsuura*'s method [20]. $[Fe^{III}(acac)_3]$ was prepared according to the method described in [21]. HPLC-grade solvents were used which have to be free of oxygen and kept under Ar.

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