130. Reaction of 5,6,7,8-Tetrahydropterin with Iron(III) Acetylacetonate. Detection of Radical Cations by Electrospray Ionization Mass Spectrometry[1])

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(2.V.95)

The experimental conditions developed for the detection of rather stable radical cations in solution by electrospray-ionization mass spectrometry (ESI-MS) of a Fe^{II} complex of 2-amino-5,6,7,8-tetrahydro-5-methylpteridin-4(3*H*)-one (**1c**) are used to observe the formation of the more unstable radical cations formed from 2-amino-5,6,7,8-tetrahydropteridin-4(3*H*)-one (**1a**) and tris(pentane-2,4-dionato)iron(III) ([Fe^{III}(acac)$_3$]; **4**) and to monitor their oxidation to the corresponding *p*-quinonoid dihydropterin complexes. These results contribute to the understanding of the important role played by 6β-5,6,7,8-tetrahydro-L-biopterin (**1b**; a homologue of **1a**) together with iron as constituent of some cofactors. The complexes obtained from **1a** and iron may be considered, *e.g.* as a model of the cofactor of the phenylalanine hydroxylase. Moreover, we describe an improved synthesis of **1c**.

Introduction. – The number of enzymes known to contain a hydrogenated pterin[4]) near a metal (Fe, Cu, Mo *etc.*) center is increasing constantly [4]. One of the best known of this class of enzymes is the phenylalanine hydroxylase in which 6β-5,6,7,8-tetrahydro-L-biopterin[5]) (Thbpt; **1b**) is the coenzyme. An iron complex containing **1b** as a ligand is thought to be essential as cofactor in mammalian cells [6] and a corresponding copper complex in certain bacteria [7].

The reaction mechanism of these enzymes is far from being exactly known, because their coenzymes are extremely reactive and, therefore, the intermediates are difficult to characterize. Yet one part of reaction is established: during the enzymatic hydroxylation of phenylalanine to tyrosine, compound **1b** is oxidized to the *p*-quinonoid 6β-6,7-dihydro-8*H*-L-biopterin (**3b**) [8], a very unstable compound which finally should be enzymatically reduced, again to Thbpt (**1b**) to recover its activity. To understand the reactivity of that enzyme and especially of its coenzyme, it seems better to study first the behavior of simpler model complexes and later on to extend the obtained results to more sophisticated derivatives.

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[1]) 'Pterin Chemistry' Part 96, presented at the 10th International Symposium on Chemistry and Biology of Pteridines, held March 21–26, 1993, in Orange Beach, Alabama, USA [1]; Part 94 [2]; Part 95 [3].

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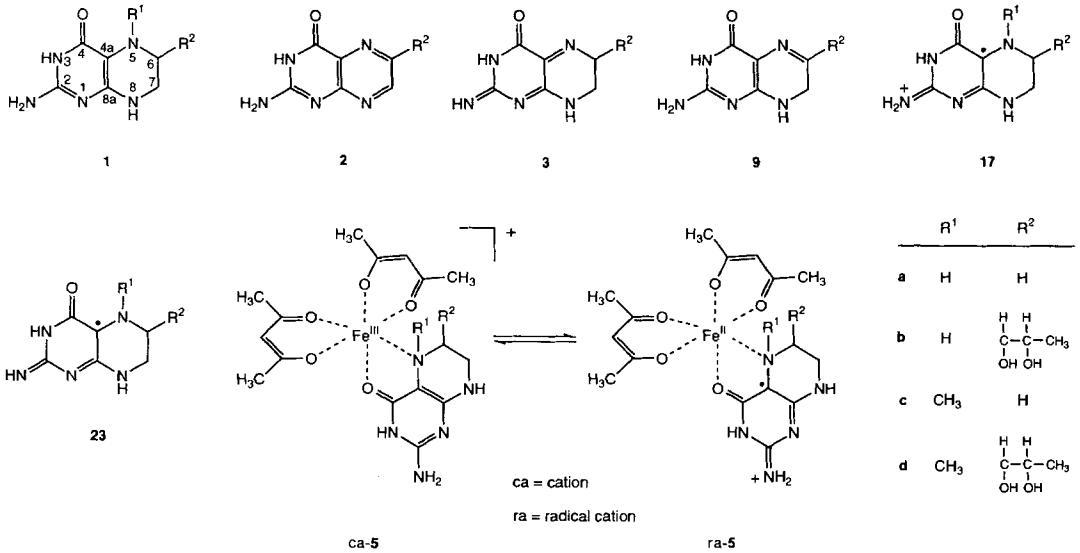
[4]) Trivial name; name according to the IUPAC rules: pterin = 2-aminopteridin-4(3*H*)-one (**2a**) and 2-amino-4-hydroxy-pteridin (enol form), respectively.

[5]) Name assigned according to the designation rules proposed in [5]. Name according to the IUPAC rules: (1′*R*,2′*S*,6*R*)-2-amino-6-(1′,2′-dihydroxypropyl)-5,6,7,8-tetrahydropteridin-4(3*H*)-one (**1b**), shortened to tetrahydrobiopterin (Thbpt).



In our search for a suitable model complex consisting of tetrahydropterin and iron, we replaced the very unstable and expensive Thbpt (**1b**) by the more stable 2-amino-5,6,7,8-tetrahydro-5-methylpteridin-4(3*H*)-one (= 5-methyl-5,6,7,8-tetrahydropterin = 5-Me-Thpt; **1c**), and we used as iron complex tris(pentane-2,4-dionato)iron(III) ($[Fe^{III}(acac)_3]$; **4**) [2]. Studying the behavior of both **1c** and **4**, we learned that electrospray-ionization mass spectrometry (ESI-MS) is a new and efficient tool to examine the reaction mechanisms in solution. Using this technique, we observed the formation of stable Fe-complexes, namely the cationic complex $[Fe^{III}(acac)_2(5\text{-Me-Thpt})]^+$ (ca-**5c**[6])) and the radical-cation complex $[Fe^{II}(acac)_2(5\text{-Me-Thpt})]^{+\cdot}$ (ra-**5c**) existing in an equilibrium, both at *m/z* 435. In addition to these two complexes, we also could show the presence of the radical cation $[5\text{-Me-Thpt}]^{+\cdot}$ (*m/z* 181, **17c**) in solution [2]. However, contrary to Thbpt (**1b**), 5-Me-Thpt (**1c**) could not be oxidized to the corresponding *p*-quinonoid dihydropterin because the Me group at N(5) cannot be eliminated as easy as an H-atom.

We, therefore, looked for a less stable tetrahydropterin derivative. We knew that for the hydroxylation of phenylalanine, Thbpt (**1b**) can be replaced *in vitro* by tetrahydropterin[7]) (Thpt; **1a**) [9] [10]. So it was of great interest to study the behavior of **1a** under the same conditions as 5-Me-Thpt (**1c**).

Results and Discussion. – *ESI-MS of the Reaction Mixture from Thpt* (**1a**) *and* $[Fe^{III}(acac)_3]$ (**4**). The ESI-MS given in *Fig. 1, a*, can be observed *ca.* 1 min after mixing Thpt·2 HCl (**1a**·2 HCl) in MeOH/H_2O 9:1 with an equimolar solution of $[Fe^{III}(acac)_3]$ (**4**). Within minutes, the spectrum changes (after 15 min, the spectrum of *Fig. 1, b*, is observed). In particular, the relative intensities of the signals at *m/z* 421/419, 322/320, 255/254, and 168/166 indicate a rapid oxidation process in the mixture due to a change of

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[6]) The prefix distinguishes the cation (ca) from the radical cation (ra) of the corresponding complexes with identical mass to charge ratio *m/z*.

[7]) Name according to IUPAC rules: tetrahydropterin (Thpt) = 2-amino-5,6,7,8-tetrahydropteridin-4(3*H*)-one.

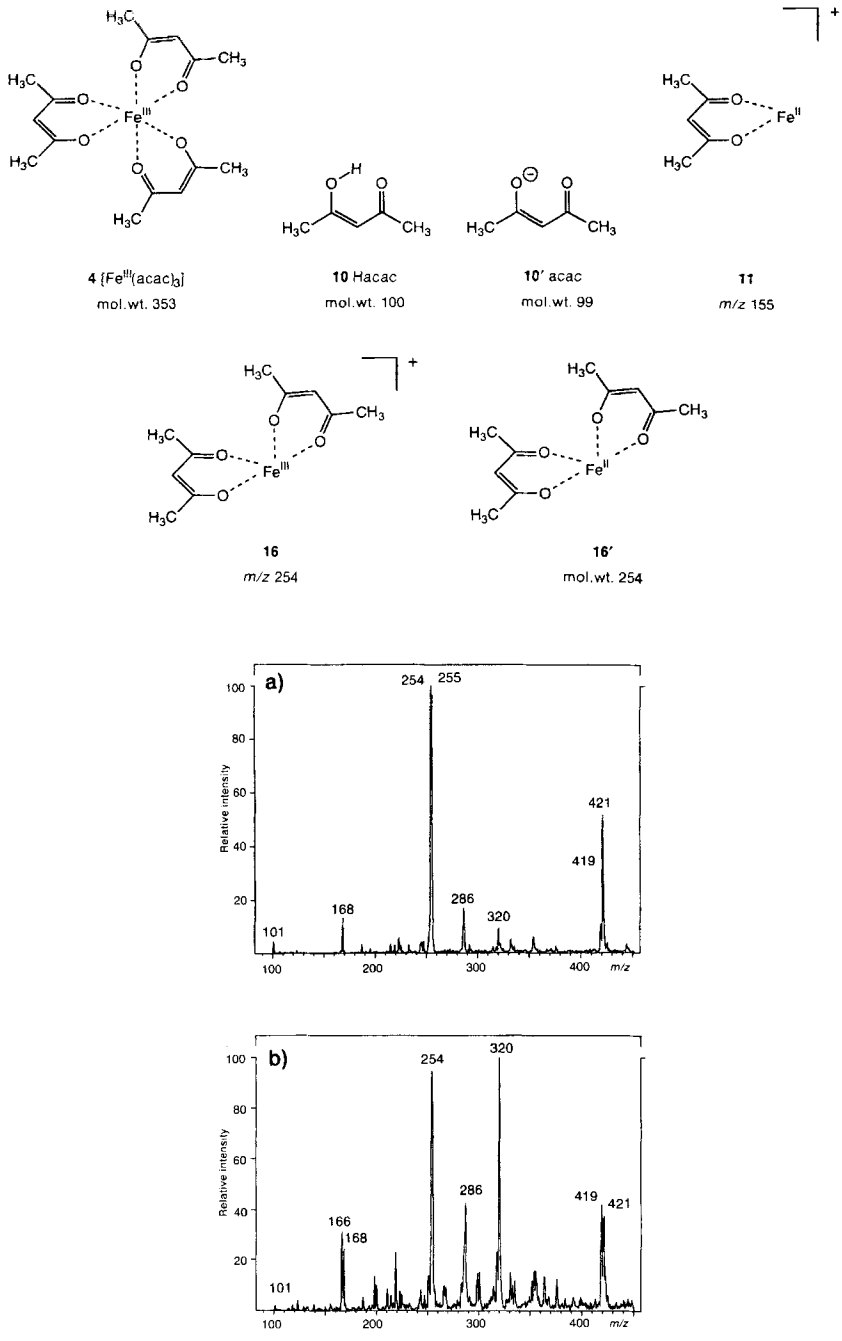


Fig. 1. a) *ESI-MS of the reaction mixture immediately after mixing Thpt·2 HCl* (**1a**·2 HCl; 10^{-4} M in MeOH/H_2O 9:1) *with an equimolar solution of* $[Fe^{III}(acac)_3]$ (**4**; 10^{-4} M in MeOH/H_2O 9:1) *and* b) *ESI-MS of the reaction mixture* **1a**/**4** (see *a*)) *after 15 min*

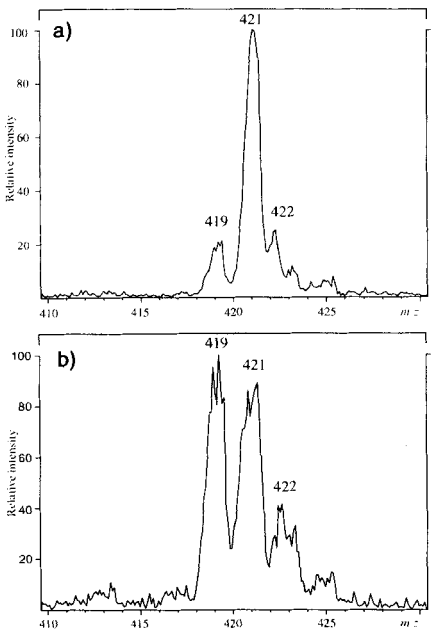


Fig. 2. *Enlargements of the section at* m/z *410–430 of* Fig. 1: a) *immediately after mixing and* b) *after 15 min*

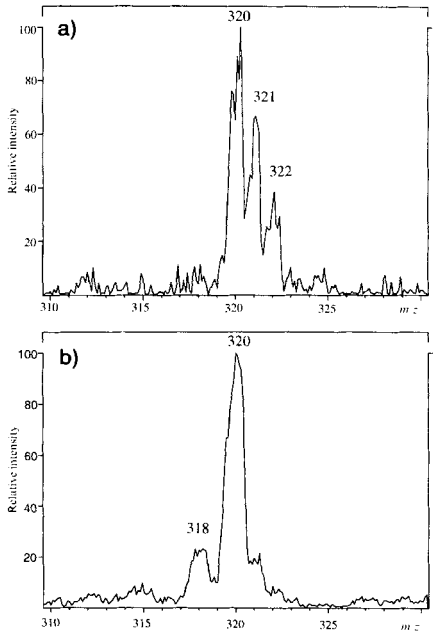


Fig. 3. *Enlargements of the section at* m/z *310–330 of* Fig. 1: a) *immediately after mixing and* b) *after 15 min*

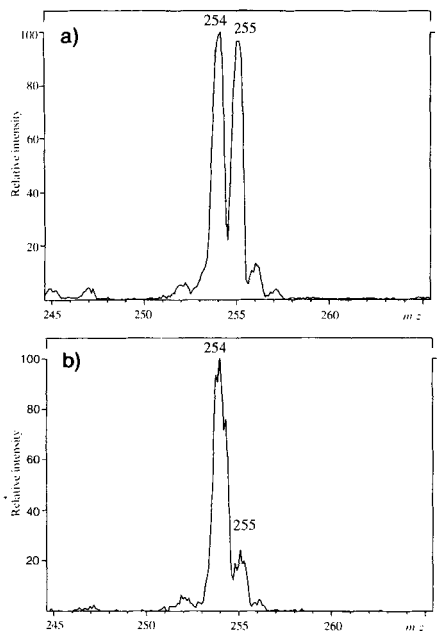


Fig. 4. *Enlargements of the section at* m/z *245–265 of* Fig. 1: a) *immediately after mixing and* b) *after 15 min*

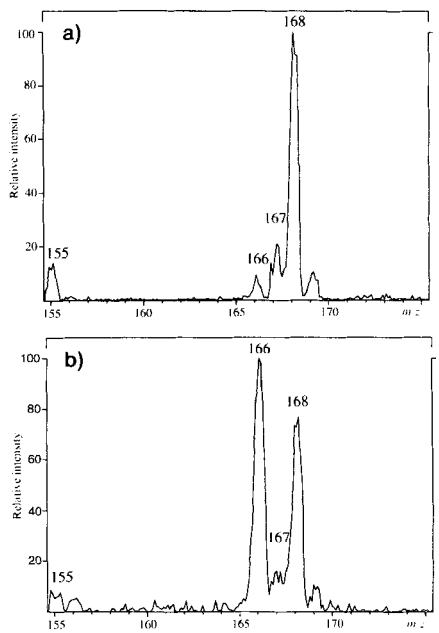


Fig. 5. *Enlargements of the section at* m/z *155–175 of* Fig. 1: a) *immediately after mixing and* b) *after 15 min*

Table. *ESI-MS Fragments of the Mixture of* **1a**·*2 HCl and* **4** *in MeOH/H_2O 9:1* (see *Figs. 1–5* and *Scheme 1*).

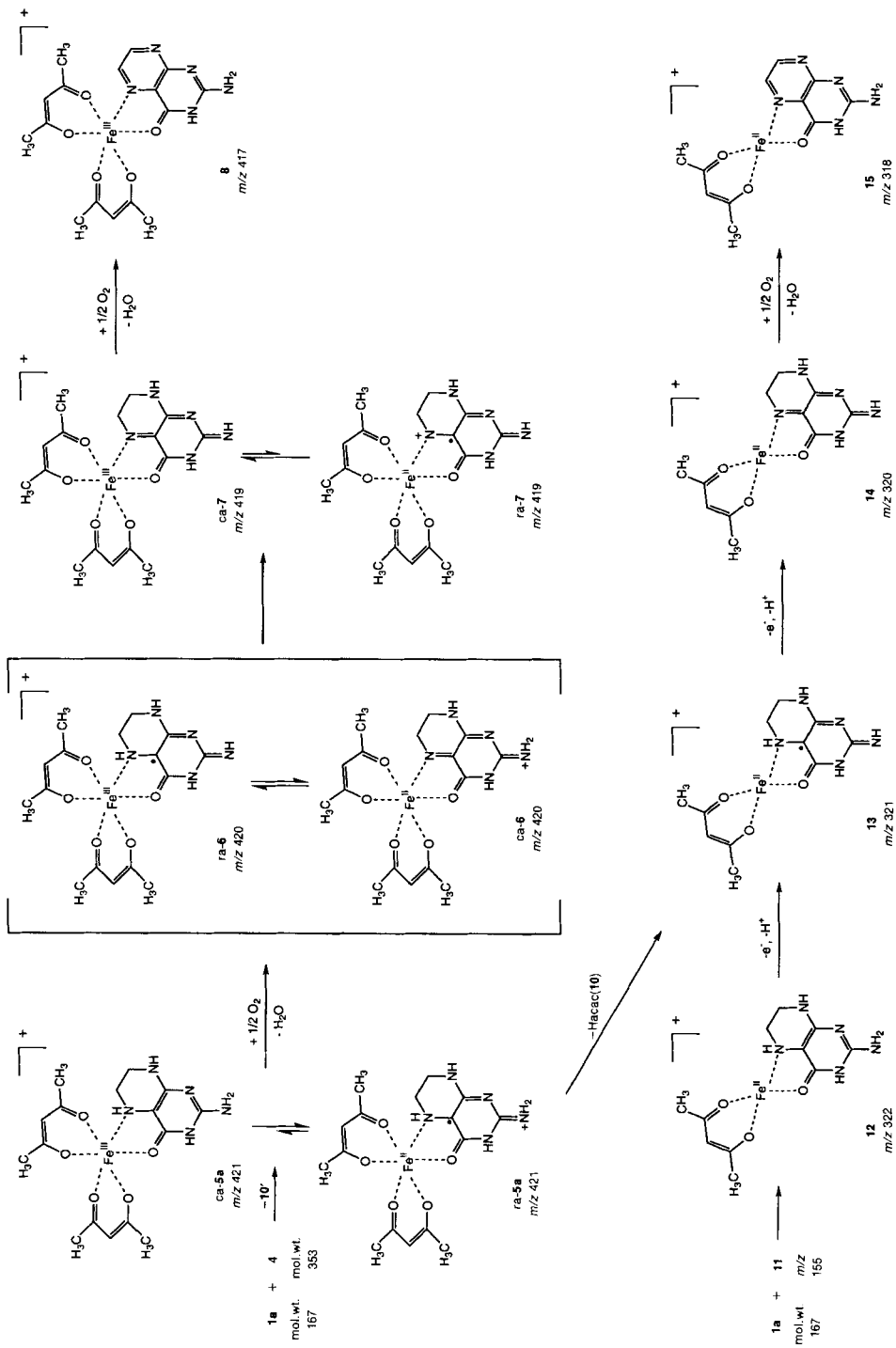
m/z	Fragment
101	$[Hacac + H]^+$ ($[\mathbf{10} + H]^+$)
155	$[Fe^{II}(acac)]^+$ (**11**)
166	[(*p*-quinonoid 6,7-dihydro-8*H*-pterin) + $H]^+$ ($[\mathbf{3a} + H]^+$) and [7,8-dihydropterin + $H]^+$ ($[\mathbf{9a} + H]^+$), resp.
167	$[Thpt]^{+\cdot}$ (**17a**)
168	$[Thpt + H]^+$ ($[\mathbf{1a} + H]^+$)
254	$[Fe^{III}(acac)_2]^+$ (**16**)
255	$[Fe^{II}(acac)_2 + H]^+$ ($[\mathbf{16'} + H]^+$)
286	$[Fe^{III}(acac)_2 + MeOH]^+$ ($[\mathbf{16} + MeOH]^+$)
318	$[Fe^{II}(acac)(pterin)]^+$ (**15**)
320	$[Fe^{II}(acac)$(*p*-quinonoid 6,7-dihydro-8*H*-pterin)$]^+$ (**14**)
321	$[Fe^{II}(acac)(Thpt)]^{+\cdot}$ (**13**)
322	$[Fe^{II}(acac)(Thpt)]^+$ (**12**)
354	$[Fe^{III}(acac)_3 + H]^+$ ($[\mathbf{4} + H]^+$)
419	$[Fe^{III}(acac)_2$(*p*-quinonoid 6,7-dihydro-8*H*-pterin)$]^+$ (ca-**7**) and $[Fe^{II}(acac)_2$(*p*-quinonoid 6,7-dihydro-8*H*-pterin)$]^{+\cdot}$ (ra-**7**), resp.
420	$[Fe^{II}(acac)_2$(*p*-quinonoid 6,7-dihydro-8*H*-pterin) + $H]^+$ ($[$ca-**6** + $H]^+$) and $[Fe^{III}(acac)_2$(*p*-quinonoid 6,7-dihydro-8*H*-pterin)$]^{+\cdot}$ (ra-**6**), resp.
421	$[Fe^{III}(acac)_2(Thpt)]^+$ (ca-**5a**) and $[Fe^{II}(acac)_2(Thpt)]^{+\cdot}$ (ra-**6a**), resp.

the oxidation number of the Fe-atom together with a loss of H-atoms from Thpt. We should emphasize that in the sample handling, a lot of care was taken to exclude O_2, but traces are still present, and they are sufficient to cause this oxidation. *Figs. 2–5* correspond to the enlargements of different sections of the spectra given in *Fig. 1*, and we assign the observed signals as shown in the *Table* and *Scheme 1*.

The spectra given in *Fig. 2* represent the enlargement of the section at *m/z* 410–430 of *Fig. 1*. $[Fe^{III}(acac)_2]^+$, present as the free complex in a solution of $[Fe^{III}(acac)_3]$ (**4**) in MeOH, and Thpt (**1a**) combine to give **5**, a mixture of the cation complex ca-**5a** ($[Fe^{III}(acac)_2(Thpt)]^+$) and the radical complex ra-**5a** ($[Fe^{II}(acac)_2(Thpt)]^{+\cdot}$; see *Scheme 1*). Both complexes with an unknown proportion of ca-**5a**/ra-**5a** are observed by ESI-MS as one signal at *m/z* 421. As mentioned already, this mixture is extremely sensitive to O_2; small amounts of molecular O_2 are in this case already sufficient for the oxidation of Thpt to a *p*-quinonoid dihydropterin ligand. Within a few minutes, a signal at *m/z* 419 can be observed, which corresponds to the fairly stable $[Fe^{III}(acac)_2$(*p*-quinonoid 6,7-dihydro-8*H*-pterin)$]^+$ cation complex ca-**7** which is in equilibrium with its radical cation ra-**7**. A small signal at *m/z* 420 (not visible in *Fig. 2*, but sometimes observed in other spectra) may represent the signal of an intermediate between the complexes **5a** (*m/z* 421) and **7** (*m/z* 419), perhaps the $[Fe^{II}(acac)_2$(*p*-quinonoid 6,7-dihydro-8*H*-pterin + H)$]^+$ ion (ca-**6**), which is in equilibrium with the unstable radical cation ra-**6**. About 1 h later, a small amount of the cation complex $[Fe^{III}(acac)_2(pterin)]^+$ (**8**) can be detected in some spectra at *m/z* 417. It has to be mentioned that the oxidation of the tetrahydropterin/iron complex **5a** to the *p*-quinonoid dihydropterin/iron complex **7** occurs within minutes, whereas the oxidation of **7** to complex **8** takes hours.

These important results allow a hypothetical explanation of the enzymatic hydroxylation of phenylalanine and other metabolites with **1b** and iron complexes as cofactors: the quinonoid compound **3b**, which is formed during these hydroxylation reactions, can be stabilized as a ligand in the iron complex and thus protected against rearrangement to **9b**

Scheme 1. *General Reaction Scheme of* **1a** *and* **4** *in Solution*



(see above for *Formula*) and further oxidation to **2b**. Therefore, it can be recycled, under *in vivo* conditions, by the dihydropterin reductase (NADH as cofactor) to **1b** and act again as cofactor.

The enlargement of the region at *m/z* 310–330 of *Fig. 1* is given in *Fig. 3*. The four signals at *m/z* 322/321/320/318 correspond to the four signals at *m/z* 421/420/419/(417) in *Fig. 2*. They show the loss of one of the acetylacetonate ligands as Hacac (**10**) from the complexes ra-**5a**. In analogy to the redox process of **5a** discussed above, the Thpt ligand in complex **12** (formed from **1a** and **11**) gives the $[Fe^{II}(acac)(p\text{-quinonoid dihydropterin})]^+$ cation **14** (*m/z* 320) *via* the radical complex **13** (*m/z* 321; *Scheme 1*). The signal at *m/z* 318 corresponds to the iron complex **15**.

Fig. 4 displays the enlargement of the section *m/z* 245–265 of *Fig. 1*. In these spectra, the redox reaction between Fe^{III} and Fe^{II} (**16**, **16'** (see above for *Formulae*): mol. wt. 254) is reflected. The dissociation of the complex ca-**5a** as well as that of ca-**7** leads to the formation of the cation **16** ($[Fe^{III}(acac)_2]^+$) showing a signal at *m/z* 254. The dissociation of the Fe^{II} radical complexes ra-**5a** and of ra-**7** leads to the neutral complex $[Fe^{II}(acac)_2]$ (**16**; mol wt. 254), which, in solution, is protonated and detected at *m/z* 255. This proves that a redox reaction occurs between Thpt and the central Fe-atom in the mixtures of the complexes **5a** and **7**.

The enlargement of the section *m/z* 155–175 of *Fig. 1* is seen in *Fig. 5*. *Fig. 5, a*, shows at *m/z* 168 the protonated form of the starting compound **1a** (mol. wt. 167). *Fig. 5, b*, displays the transformation of **1a** to the radical cation **17a** (see above for *Formula*) which is observed as a weak signal at *m/z* 167, and the oxidation to the dihydropterin (**3a**/**9a**) which shows, after protonation, a signal at *m/z* 166. An extremely weak signal of the pterin (**2a**), a compound which is not easily protonated and detected by ESI-MS, may be observed at *m/z* 164 in some spectra. Numerous ESI-MS of the reaction between **1a** and **4** show that the velocity of the formation of **3a** is strongly dependent on how much care is taken to exclude O_2 from the reaction mixture (degassing of the solvents *etc.*). We propose in *Scheme 1* a reaction mechanism which leads to ligand **3a** coordinated to the Fe-atom in the complexes ca-**7** and **14**.

Confirmation of Complex Structures by Electrospray-Ionisation Tandem Mass Spectrometry (ESI-MS/MS). The ESI tandem mass spectrometry (ESI-MS/MS) allows to study the fragmentation pathway in the gas phase of a compound detected in the ESI-MS.

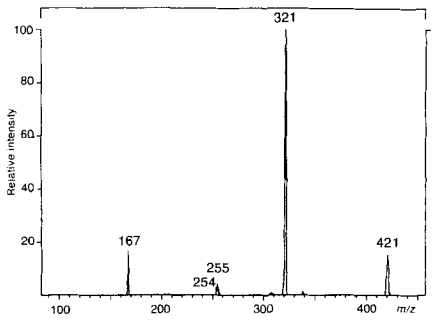


Fig. 6. *ESI-MS/MS on ion* m/z *421* (ca-**5a** and ra-**5a**) *formed by addition of* **1a** *and* **4**. Conditions, see *Fig. 1* (collision offset −10; collision gas: Ar, 3 mTorr).

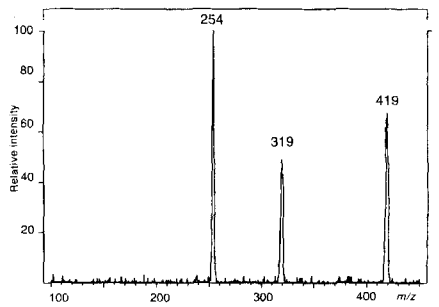


Fig. 7. *ESI-MS/MS on ion* m/z *419* (ca-**7** and ra-**7**) *formed by addition of* **1a** *and* **4**. Conditions, see *Fig. 1* (collision offset −10; collision gas: Ar, 3 mTorr).

Charged fragments are detected directly, neutral fragments formed give no signal in ESI-MS/MS. Using ESI-MS and ESI-MS/MS, the existence and the behavior of the Fe^{II} and Fe^{III} complexes in the solution and in the gas phase could be studied and compared.

Fig. 6 shows, *e.g.*, the fragmentation of complex **5a** which represents theoretically an equilibrium mixture of the two complexes ra-**5a** and ca-**5a** (ESI-MS/MS on *m/z* 421), both ra-**5a** and ca-**5a** should follow different fragmentation pathways. An explanation for the fragments formed is given in *Scheme 2*. The signals of the main fragments are at *m/z* 321 (**13**) and *m/z* 167 (**17a**). Two rather small signals can be observed at *m/z* 254 (**16**)

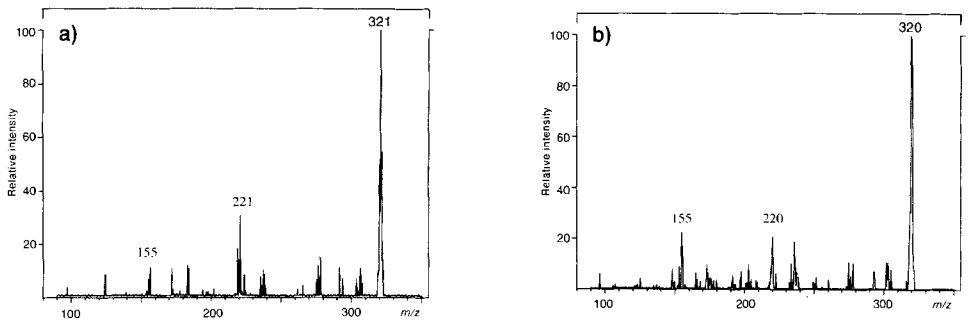
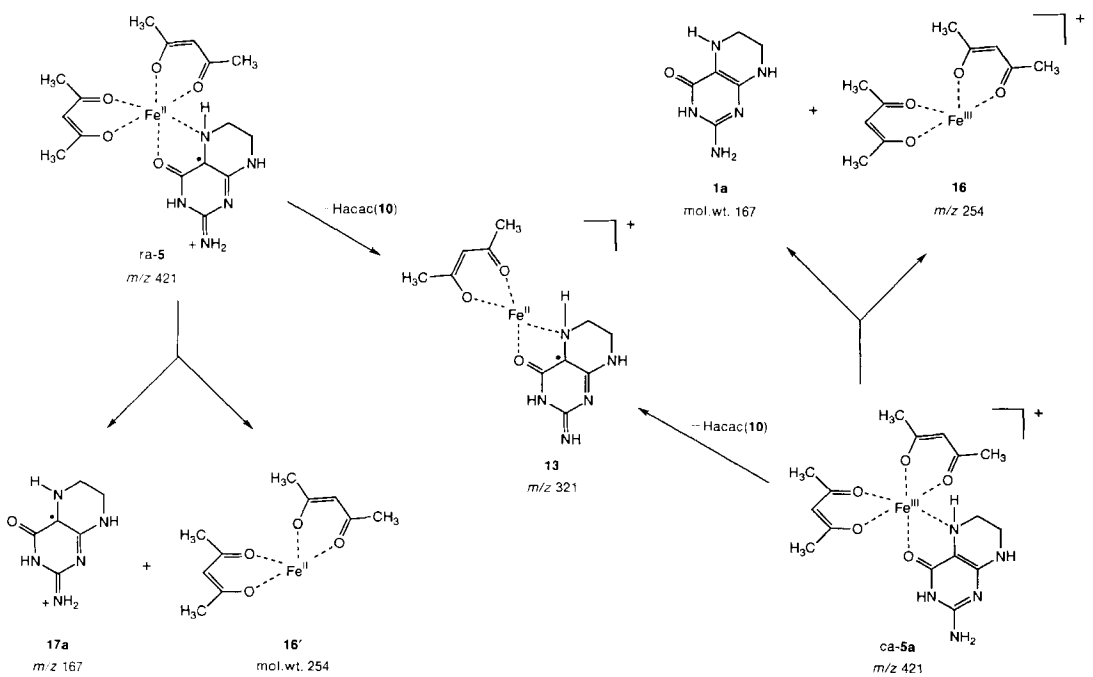


Fig. 8. *ESI-MS/MS* a) *on ion* m/z *321* (**13**) *formed by addition of* **1a** *and* **4** (conditions, see *Fig. 1* (collision offset −35; collision gas: Ar, 3 mTorr)) *and* b) *on ion* m/z *320* (**14**; details see above)

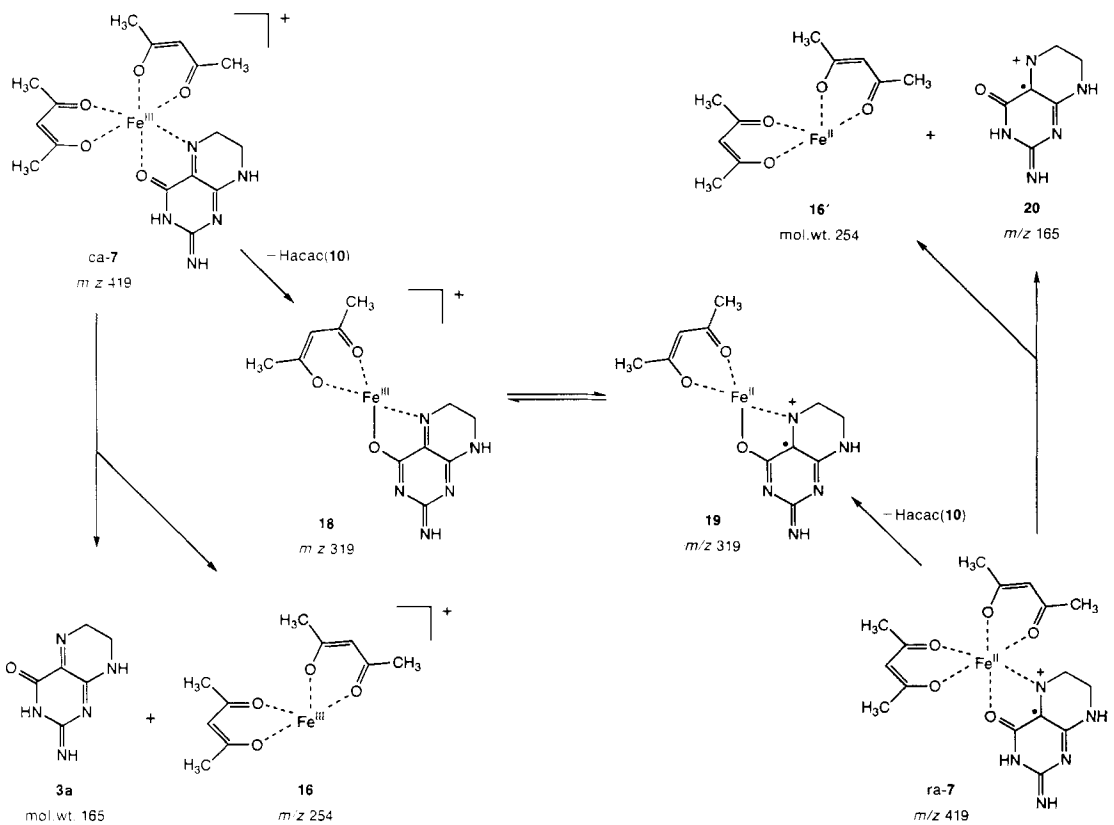
Scheme 2. *Fragmentation Pathway of Compound* ra-**5a** *and* ca-**5a** (ESI-MS/MS, *cf. Fig. 6*)



and m/z 255 ([**16′** + H]$^+$). There is a small probability for an intramolecular proton transfer from the Thpt ligand in complex ra-**5a** to the other part of the ion to form the protonated **16′**. It follows that ion m/z 321 is formed from the mother ion m/z 421 by loss of the neutral fragment **10**. This is possible from both ra-**5a** and ca-**5a**. But the fragment ion at m/z 167 (**17a**) can only be explained from ra-**5a** by loss of the neutral **16′** (mol. wt. 254). On the other hand, the charged **16** (m/z 254) is best explained by loss of neutral Thpt (**1a**) from ca-**5a**. This result proves that the signal at m/z 421 used for the tandem mass experiment is due to a mixture of the Fe^{II} complex ra-**5a** and the Fe^{III} complex ca-**5a**. The spectrum represented by *Fig. 6* is taken right at the beginning of the fragmentation reaction. The low intensity of the ion at m/z 254 demonstrates that the equilibrium between ca-**5a** and ra-**5a** is preferentially on the side of the Fe^{II} complex ra-**5a**. It seems that as soon as a complex between **1a** and **4** is formed (*cf. Scheme 1*), the Thpt ligand gives one electron to the Fe-center, and a reduction from Fe^{III} to Fe^{II} occurs. The signal at m/z 321 (**13**) is due to the Fe^{II} complex, which is formed by loss of neutral acetylacetone (**10**) from ra-**5a**.

For the ESI-MS/MS in *Fig. 7*, we chose the mass m/z 419, thus selecting the ions of the hypothetical mixture of the Fe^{III} complexes ca-**7** and the Fe^{II} complex ra-**7**. Each of them

Scheme 3. *Fragmentation Pathway of Compound* ca-**7** *and* ra-**7** (ESI-MS/MS, *cf. Fig. 7*)

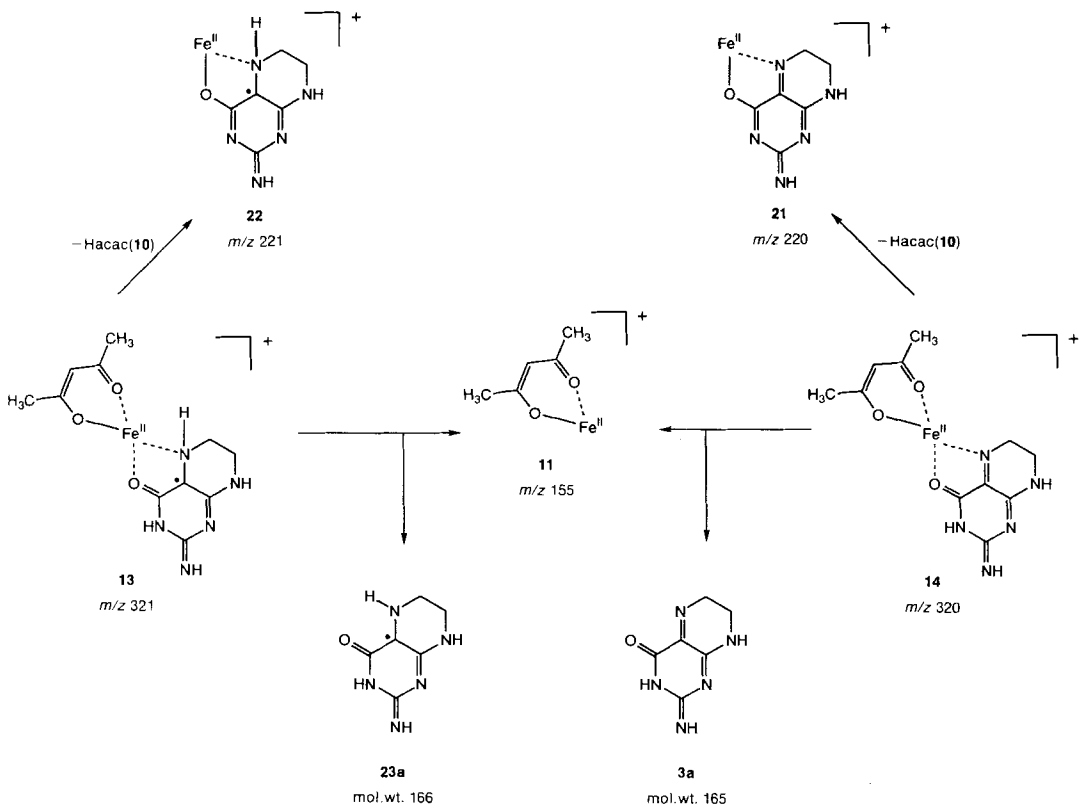


undergoes a different fragmentation pathway. Two pronounced fragment ions are registered: *m/z* 319 and 254. The ion ca-**7** (*Scheme 3*) can decompose either to the neutral **10** (Hacac, mol. wt. 100) and the charged complex **18** (*m/z* 319) which is in equilibrium with **19**, or to the charged complex **16** (*m/z* 254) and the neutral *p*-quinonoid dihydropterin **3a** (mol. wt. 165), while ra-**7** can be fragmented either into **10** (Hacac) and **19** (*m/z* 319), already formed from ca-**7**, or to the neutral complex **16'** (not detected) and to the radical cation **20** (*m/z* 165). *Fig. 7* shows only the signals at *m/z* 319 and 254, but no signal at *m/z* 165. We thus conclude that if ra-**7** exists in equilibrium with ca-**7**, the equilibrium must lie far over in favor of ca-**7** (*Schemes 1* and *3*).

In the same way, we can explain the fragmentation of the ions *m/z* 321 and 320 (*Fig. 8* and *Scheme 4*) and thus confirm the structures of the Fe^{II} radical cation **13** (*m/z* 321) and the Fe^{II} cation **14** (*m/z* 320). The Fe^{II} cation **11** is observed at *m/z* 155 besides the radical cation **22** (*m/z* 221) in *Fig. 8, a*, and besides the cation complex **21** (*m/z* 220) in *Fig. 8, b*, whereas the neutral radical **23a** (mol. wt. 166; see also *Formula* above) is of course not observed in *Fig. 8, a*, and the neutral *p*-quinonoid **3a** (mol. wt. 165) as well is not observed in *Fig. 8, b*.

To summarize, the fragmentation cascades observed in *Figs. 6–8* support the assignment of the structures which are proposed in *Scheme 1* to explain the signals found in the original spectra (*Fig. 1*, *Table*).

Scheme 4. *Fragmentation Pathway of Compound* **13** *and* **14** (ESI-MS/MS, *cf. Fig. 8*)



Confirmation of the Formation of the Radical **17a** *During the Reaction of* **1a** *with* **4**. The detection of the radical **17c** (see above for *Formula*) by ESI-MS was easy in the reaction of Fe^{III} complex **4** with 5-Me-Thpt (**1c**), because the stabilizing effect of the Me group at N(5) allows a prolonged life to **17c** [2]. In contrast, Thpt (**1a**) with an H-atom at N(5) is much more reactive, and the radical **17a** is rapidly oxidized to the *p*-quinonoid compound **3a** so that the detection of **17a** by ESI-MS is more difficult. However, the presence of **17a** can be verified by electron paramagnetic resonance (EPR) spectroscopy (*Fig. 9*). For its detection, it was necessary to mix the solutions of **1a** and **4** directly in the EPR cavity. Under these conditions, the lifetime of the free radical is *ca.* 30 s. The spectra obtained are very similar to the known EPR spectra of the tetrahydropterin radicals formed by H_2O_2 oxidation under acidic conditions [11] and to that of **17c** [2] under conditions very similar to those reported here.

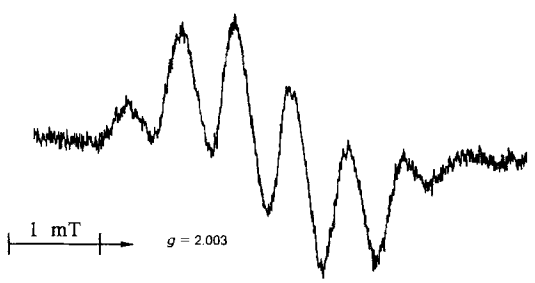


Fig. 9. *EPR Spectrum of a 1:1 mixture of THP·2 HCl* (**1a**·2 HCl) *and* $[Fe^{III}(acac)_3]$ (**4**), 10^{-2} M *each, in MeOH/*H_2O *9:1* (pH 2–3), *proving the formation of the radical* **17a**. For the detection, it was necessary to generate it directly in the EPR cavity by mixing their solutions.

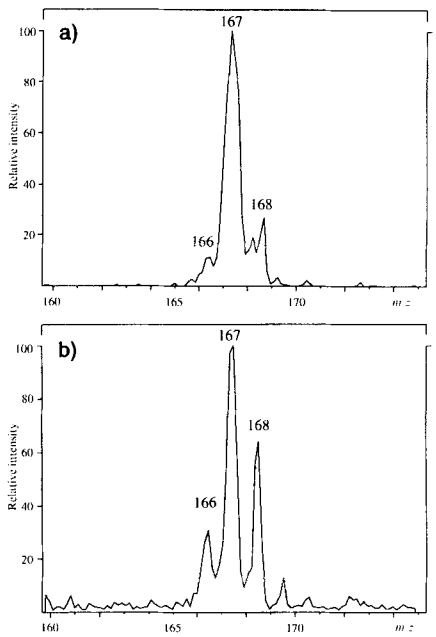


Fig. 10. *Detection of the radical* m/z *167* (**17a**) *with ESI-MS*. For reaction conditions, see *Fig. 1*, but with *ca.* 5% deficit of Thpt; extreme care was taken to exclude O_2 from the solutions. Spectrum *b* was measured *ca.* 10 min after that of *a*.

As we previously mentioned, an univocal demonstration of the presence of **17a** in a normal ESI-MS is not easy (*Fig. 6*). Nevertheless, a perfect signal of the radical cation **17a** in a normal ESI-MS spectrum is possible as seen in *Fig. 10*. In this case, a small deficit of Thpt (**1a**) is used in the reaction and, extreme care is taken to exclude O_2 during the reaction.

Oxidation Mechanisms of Thpt (**1a**). During our experiments, we noticed that over a period of time the signal-to-noise ratio was getting worse in the ESI-MS, due to the increasing number of species formed by oxidation reactions in the mixture. We always use the expression 'oxidation' to name these processes, because, according to the proposed *Scheme 1*, **1a** loses two protons and two electrons, when the *p*-quinonoid dihydropterin **3a** or its derivatives **14** and **7** are formed.

The only species which can accept electrons and protons or electrons alone, separately or at the same time under the experimental conditions, are either molecular O_2 or Fe^{III}, according to the formal *Eqns. 1* and *2*.

$$\mathbf{1a} + \tfrac{1}{2}O_2 \rightarrow \mathbf{3a} + H_2O \quad (1)$$

$$\mathbf{1a} + Fe^{III} \rightarrow \mathbf{17a} + Fe^{II} \quad (2)$$

The reaction of *Eqn. 1* may theoretically be easily developped as shown in *Eqns. 3* and *4* so that the final result is *Eqn. 5*.

$$\mathbf{1a} + \tfrac{1}{2}O_2 - 2H^+ - 2e \rightarrow \mathbf{3a} + H_2O \quad (3)$$

$$\mathbf{3a} + \tfrac{1}{2}O_2 - 2H^+ - 2e \rightarrow \mathbf{2a} + H_2O \quad (4)$$

$$\mathbf{1a} + O_2 - 4H^+ - 4e \rightarrow \mathbf{2a} + 2H_2O \quad (5)$$

In case of a Fe^{III} complex as catalyst, both reactions of *Eqns. 1* and *2* occur simultaneously, so that the redox processes and acid-base reactions take place at the same time.

On the basis of the above mentioned reactions, we propose the hypothetic reaction sequence of *Eqns. 6–8*, involving an intramolecular transfer of a proton from NH_2–C(2) to O_2 and of one electron from Fe^{II} to the proton. Then an intramolecular transfer of a hydroperoxide radical occurs from Fe^{III} to the unpaired electron at C(4a) (*Eqn. 9*), as we already proposed in [11], folowed by the reactions of *Eqns. 10–12*, resulting in the overall reaction of *Eqn. 13*.

$$\mathbf{1a} + \mathbf{4}(Fe^{III}) \rightarrow \mathbf{10'} + \text{ra-}\mathbf{5a}(Fe^{II}) \quad (6)$$

$$\text{ra-}\mathbf{5a} + O_2 \rightarrow [\text{ra-}\mathbf{5a}\text{–}O_2] \quad (7)$$

$$[\text{ra-}\mathbf{5a}\text{–}O_2] \rightarrow \text{ra-}\mathbf{24}(Fe^{III}O\text{–}O\text{–}H) \quad (8)$$

$$\text{ra-}\mathbf{24} \rightarrow \text{ca-}\mathbf{25}(Fe^{III}) \quad (9)$$

$$\text{ca-}\mathbf{25} \rightarrow \text{ca-}\mathbf{7}\,(Fe^{III}) + HO\text{–}OH \text{ or } 2\,HO\cdot \quad (10)$$

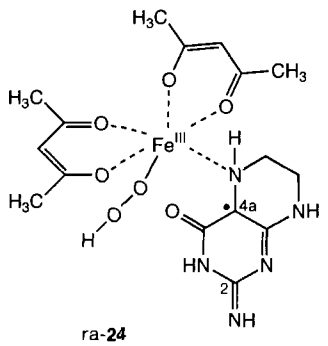
$$\text{ca-}\mathbf{7} \rightarrow \mathbf{3a} + \mathbf{16} \quad (11)$$

$$\mathbf{16} + \mathbf{10'} \rightarrow \mathbf{4} \quad (12)$$

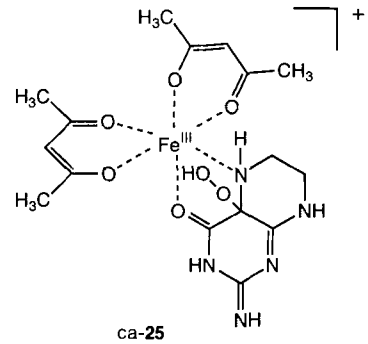
---

overall reaction $\mathbf{1a} + O_2 \xrightarrow{\mathbf{4}\,(Fe^{III})} \mathbf{3a} + HO\text{–}OH \text{ or } 2\,HO\cdot$ (13)

From this, the following conclusions can be drawn: *1*) Compound **4** acts as a catalyst only. *2*) The oxidation of Thpt to quinonoid 6,7-dihydro-8*H*-pterin **3a** needs at least one molecule of O_2. *3*) We should expect that **3a**, stabilized as ca-**7**, would have its nucleus oxidized by H_2O_2 or 2 HO· so that more than one molecule of O_2 would be necessary to oxidize Thpt to a pterin derivative. To verify this hypothesis, we measured the quantity of O_2, needed for the oxidation process of **1a** in the presence of **4** as catalyst and for the oxidation without the catalyst. The result of these experiments are shown in *Fig. 11*. They



ra-**24**



ca-**25**

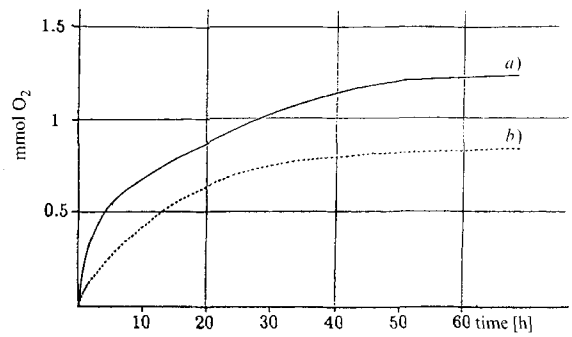


Fig. 11. *Oxidation curves of Thpt* (**1a**) *exposed to air* (10^{-3} M in dioxane/H_2O 9:1, pH 1.5–7.5, 725 Torr, 22–25°) a) *in the presence of an equimolar amount of* $[Fe^{III}(acac)_3]$ (**4**) *and* b) *without* $[Fe^{III}(acac)_3]$ *addition*

confirm the results already obtained some years ago under somewhat different conditions [9]. The following conclusions can be made:

Oxidation in the *presence* of the Fe^{III} catalyst **4** (*Fig. 11a*): *i*) The reaction does not follow the stoichiometric *Eqns. 3* and *4*. More than 1 mol of O_2 is used for this oxidation. *ii*) The activation of O_2 in the presence of Fe complexes is quite complicated. Besides a small quantity of pterin **2a**, numerous other compounds are detected in the solution by TLC at the end of the oxidation. *iii*) The labile compound **3** is to some extend stabilized against further oxidation in the complexes **14** or **7** with iron and acetylacetonate. The oxidation curve shows a bend after the reaction of 1 atom of O_2, from that point on, the velocity of the oxidation is reduced.

Oxidation in the *absence* of the Fe^{III} catalyst **4** (*Fig. 11b*): *i*) The rate of reaction is slower. The mechanism of the oxidation must differ from the one observed in *Fig. 11a*: *ii*) Less than 1 mol of O_2 is necessary for the oxidation, **1a** → **2a**. The reaction seems to follow *Eqns. 3* and *4*. Pterin (**2a**) alone is detected in the reaction solution by TLC. *iii*) If **3a** is the first oxidation product which is formed, and if it is not stabilized in a complex, it rearranges rapidly to compound **9a** as it is well established by UV spectroscopy [12]. Compound **9a** is oxidized to pterin (**2a**) by an unknown reaction mechanism. The observed oxidation curve in *Fig. 11b* displays the expected smooth form.

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Experimental Part

General. Chemicals: *Celite® 535*, PtO_2 (*Adams* catalyst), methanal (36% aq. soln.), solvents *puriss.*, all from *Fluka*, CH-9470 Buchs. TLC: silica gel *Merck 60* F_{257}, eluent i-PrOH/3% aq. H_3BO_3 4:1. NMR Spectra: *Varian-XL-200* spectrometer. EPR Spectrum: *Bruker ESP 300*. ESI-MS: *Finnigan-TSO-700* spectrometer equiped with an electrospray ion source; solvents free of O_2; for standard ionization conditions, see *Figures*.

One-Pot Synthesis of 5-Methyl-5,6,7,8-tetrahydropterin Dihydrochloride (**1c**·2 HCl). This is a modified and improved general method developed by *Rylander* [13], *Matsuura* and *Sugimoto* [14], and *Bosshard et al.* [15]. At 22°, $PtO_2 \cdot 4\,H_2O$ (100 mg, *ca.* 0.35 mmol) in MeOH (120 ml), H_2O (30 ml), and 1N aq. HCl (6 ml) was prehydrogenated (24 ml of H_2O consumed at 740 Torr). Then the pterin (**2a**; 330 mg, 1.8 mmol) was added under N_2, and the hydrogenation was continued. It took 10 h until the blue fluorescence of the soln. vanished (67 ml of H_2 at 22°/740 Torr). After flushing the flask with N_2, methanal (0.85 ml, 10 mmol) was added to the soln. of the formed **1a**, and the hydrogenation was continued for 3 h (33 ml of H_2 at 22°/740 Torr). Then H_2 was replaced by N_2. After 1 h, Pt was settled, and the colorless supernatant was decanted. The remaining catalyst was filtered off and the clear filtrate added to the supernatant. The combined soln. was evaporated, the resulting slurry mixed well with dry EtOH (*ca.* 30 ml), the mixture evaporated, and the solid dissolved in MeOH (35°). The small amount of Pt remaining in this soln. was removed by adding charcoal, filtering over a glass filter (*D4*) covered with *Celite® 535*, and rinsing with MeOH (10 ml). To this soln., first the same amount of anh. EtOH was added followed by dry Et_2O until the compound precipitated. The precipitate was filtered (*D4* glass filter) off, washed with a few ml of dry EtOH/Et_2O 1:1 and dried over NaOH pellets (22°/15 Torr): 350 mg (75%) of **1c**·2 HCl. The crude product is unstable, hygroscopic, and easily oxidized by air to pterin (TLC). After recrystallization (1N HCl/EtOH), **1c**·2 HCl was kept under dry N_2 to protect it from O_2 and moisture. UV: analogous to the UV of Thpt (**1a**) [16] and 5-methyl-tetrahydro-L-biopterin (**1d**) [15]. ^{1}H-NMR (0.1N DCl/D_2O): 3.87 (*t*, H_{eq}–C(7), H_{eq}–C(6)); 3.64 (*t*, H_{ax}–C(7), H_{ax}–C(6)); 3.20 (*s*, Me–N(5)). Anal. calc. for $C_7H_{13}Cl_2N_5O$ (254.118): C 33.09, H 5.16, Cl 27.90, N 27.56; found: C 32.96, H 5.37, Cl 27.21, N 27.16.

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