Elimination of Ammonium Ion from the α-Hydroxyalkyl Radicals of Serine and Threonine in Aqueous Solution and the Difference in the Reaction Mechanism

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Dedicated to Professor Dr. Dietrich Schulte-Frohlinde *on the Occasion of his 60th Birthday*

Elimination of NH_4^+ Ion, Serine, Threonine, Radical Decay, Amino Acids

The zwitterionic radicals $HO-\dot{C}H-CH(COO^-)NH_3^+$ (**4a**) and $HO-\dot{C}(CH_3)-CH(COO^-)NH_3^+$ (**4b**) are the main species produced upon $OH^\cdot$ radical attack in aqueous solutions at pH 3–7 at the amino acids serine, $HO-CH_2-CH(COO^-)NH_3^+$, or threonine, $HO-CH(CH_3)-CH(COO^-)NH_3^+$, respectively. Both radicals undergo elimination of NH_4^+ ion to form the radicals $O=CH-\dot{C}H-COO^-$ (**7**) or $CH_3-CO-\dot{C}H-COO^-$ (**9**) respectively.

The pK_a of the serine-derived cationic radical $HO-\dot{C}H-CH(COOH)NH_3^+$ (**3a**) (**3a** ⇄ **4a** + H^+), was determined by ESR spectroscopy to 2.2 ± 0.1 at 276 K. From kinetic data the $pK_a(OH)$ of radical **4a** (**4a** ⇄ $^-O-\dot{C}H-CH(COO^-)NH_3^+$ (**5a**) + H^+) was calculated to 7.0. The elimination of NH_3 takes place from the ketyl radical **5a** (*type-B* mechanism), the rate constant was calculated from kinetic data to $2.4 \times 10^6\ s^{-1}$ at 290 K.

The half-lives of radicals **4a** and **4b** were measured by time-resolved conductivity changes upon pulse radiolysis, 170 ± 10 μs for **4a** and 26 ± 2 μs for **4b**, at 290 K and pH 5.8.

With the threonine derived radicals elimination of NH_3 takes place at the stage of the α-hydroxyalkyl radical **4b** (*type-A* mechanism). In this series the pK_a of the product radical $CH_3-CO-\dot{C}H-COOH$ (**8**) (**8** ⇄ **9** + H^+), was determined by ESR spectroscopy to 2.7 ± 0.1.

The reasons for the observed mechanistic differences (*type-A versus type-B* decay) are discussed. As further examples for a *type-B* decay some preliminary data on the elimination of HF from the radicals $CF_3-\dot{C}(OH)-CF_3$ and $CF_3-\dot{C}H-OH$ have been added.

Introduction

α-Hydroxyalkyl radicals which carry a leaving group X in the β-position (X = F, Cl, Br, $OCOCH_3$, OPO_3H^-) are known to undergo heterolytic decay in aqueous solution (ref. [1, 2] and references cited in [2]). Reaction (1) is an example.

$$HO-\dot{C}H-CH_2-X \rightarrow H^+ + O=CH-\dot{C}H_2 + X^- \quad (1)$$

With the above leaving groups the reactions are that fast that the α-hydroxyalkyl radicals cannot be observed by ESR spectroscopy in aqueous solution ($k_1 = 5 \cdot 10^5\ s^{-1}$ at room temperature for X = $OCOCH_3$ and is still larger with X = halogens [2]). Mechanistic details will be referred to in the Discussion.

α-Hydroxyalkyl radicals with "bad" leaving groups* like *e.g.* OH or OCH_3 in β-position are observable as such in close to neutral aqueous solution by ESR spectroscopy. In order to detect their heterolytic decay acid or base catalysis is required [3–5], for examples see reactions (2) and (3). Reactions of this type have frequently been discussed in radical chemistry of sugars and related compounds [6].

$$HO-\dot{C}H-CH_2-OH + H^+ \rightleftarrows HO-\dot{C}H-CH_2-OH_2^+ \quad (2a)$$

$$HO-\dot{C}H-CH_2-OH_2^+ \rightarrow H^+ + O=CH-\dot{C}H_2 + H_2O \quad (2b)$$

$$HO-\dot{C}H-CH_2-OH + OH^- \rightarrow {}^-O-\dot{C}H-CH_2-OH + H_2O \quad (3a)$$

$${}^-O-\dot{C}H-CH_2-OH \rightarrow O=CH-\dot{C}H_2 + OH^- \quad (3b)$$

The acid catalysis improves the leaving group propensity (decreases the nucleophilicity of the leav-

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* Leaving group propensity is a relative figure and should always be seen in context with the electrophilicity of the organic frame from which departure takes place.

ing group) and the base catalysis reduces the electrophilicity of the organic skeleton*.

α-Hydroxyalkyl radicals with NH_3^+ groups in β-position or with alkylated such groups, NR_3^+, respectively, are known from ESR spectroscopy to decay without catalysis at pH 6.5 but to gain stabilization towards that decay at pH 2. This phenomenon was mechanistically explained [8] by assuming dissociation of H^+ from the OH group of radical **1** to give the ketyl radical **2**, reaction (4f). The ketyl radical **2** then decays with formation of NH_3 and an acylalkyl radical which was the species observed at pH 6.5, reaction (4d). At pH 2, however, reprotonation of the ketyl **2** to give radical **1**, reaction (4r), became that much faster than reaction (4d) that only radical **1** was observed.

$$\underset{\mathbf{1}}{HO-\dot{C}R-CH_2-NH_3^+} \underset{(4r)}{\overset{(4f)}{\rightleftarrows}} H^+ + \underset{\mathbf{2}}{{}^-O-\dot{C}R-CH_2-NH_3^+} \qquad (4f), (4r)$$

$$\underset{\mathbf{2}}{{}^-O-\dot{C}R-CH_2-NH_3^+} \xrightarrow[(4d)]{} O{=}CR-\dot{C}H_2 + NH_3 \qquad (4d)$$

1a, **2a**: R = H, **1b**, **2b**: R = CH_3

C-N bond breaking of the ketyl radical in reaction (4d) is analogous to the C_β-O bond breaking of the ketyl radical in reaction (3b), but the formation of the ketyl radical by reaction (4f) is spontaneous while formation of the ketyl radical in reaction (3a) is by reaction with base.

α-Hydroxyalkyl radicals derived by H-abstraction by $OH^\cdot$ radicals in aqueous solution from the amino acids serine or threonine, respectively (*cf.* Scheme 1 and Scheme 2), could be expected to belong into the same class as the α-hydroxy-β-ammonioalkyl radicals and to decay analogously. ESR spectra of radicals **3a** and **3b** (measured at pH 2) [10, 11] and of radical **4a** (measured at pH 6.5) [10, 12], see Scheme 2, have been reported and the formation of these radicals by $H^\cdot$-abstraction has been confirmed by the observation of spin adduct radicals [13]. The decay of these radicals, however, has not been reported so far.

Radical **4b** was not observed by ESR spectroscopy. With threonine the formation of radical $\dot{C}H_2-CH(OH)-CH(COOH)NH_3^+$ is documented [10, 11, 13]. The assignment to cationic or zwitterionic radicals **3** or **4** was based on the assumption that the pK_a's of the radicals were not too far apart from those of the parent compounds.

We have now been able to measure the pK_a of radical **3a** and to produce evidence for the decay of the zwitterionic radicals **4a** and **4b**. We used ESR spectroscopy, time resolved electrical conductivity measurements in combination with pulse radiolysis and product analysis after ^{60}Co-γ-radiolysis. We shall show that only radical **4a** decays through formation of its ketyl radical **5a** analogously as **1a** does *via* **2a** (see reactions (4)) but that radical **4b** decays by rate

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* Heterolytic cleavage reactions may be regarded as intramolecular redox reactions. From this viewpoint acid catalysis improves the oxidative power of the leaving group and base catalysis improves the reducing power of the radical site which latter is in accord with measured such properties of ketyl radicals compared with α-hydroxyalkyl radicals [7]. Both kinds of explanation, however, are equivalent and both will be used in that sense throughout this paper.

$$\underset{\text{serine}}{HO-CH_2-CH(COO^-)NH_3^+} \qquad \underset{\text{threonine}}{HO-CH(CH_3)-CH(COO^-)NH_3^+}$$

pK_a's at 274 K [9]:

2.3 (COOH), 9.9 (NH_3^+) ; 2.2 (COOH), 9.7 (NH_3^+)

Scheme 1. The amino acids under investigation and their pK_a values at 274 K.

$$\underset{\mathbf{3}}{HO-\dot{C}R-CH(COOH)NH_3^+} \qquad \underset{\mathbf{4}}{HO-\dot{C}R-CH(COO^-)NH_3^+}$$

$$\underset{\mathbf{6}}{{}^-O-\dot{C}R-CH(COOH)NH_3^+} \qquad \underset{\mathbf{5}}{{}^-O-\dot{C}R-CH(COO^-)NH_3^+}$$

3a, **4a**, **5a**, **6a**: R = H, radicals derived from serine,
3b, **4b**, **5b**, **6b**: R = CH_3, radicals derived from threonine.

Scheme 2. α-Hydroxylalkyl or ketyl radicals in different ionization states derived from serine or threonine, respectively, with the numbering used in this paper.

determining C-N bond breakage and rather belongs into the first mentioned class of radicals (see reaction (1)). In view of the close similarity of radicals **4a** and **4b** this result is quite unexpected. The factors which determine the selection of the mechanism of decay of α-hydroxyalkyl radicals will be discussed.

Experimental

Materials

Serine and threonine (Merck) were used as received. 2-Aminoethanol and 1-amino-propanol-2 (EGA) were vacuum-distilled prior to use. Water was triply distilled. Other chemicals were of analytical grade.

ESR spectroscopy

A photolytic *in situ* technique was used [2, 14]. $OH^\cdot$ radicals were produced by photolysis of H_2O_2. Optimal signals were obtained at 0.1 M H_2O_2. The reaction temperature was 276 K, pH values were adjusted with $HClO_4$ or NaOH.

^{60}Co-γ-radiolysis

In water $OH^\cdot$ radicals, $H^\cdot$ atoms and solvated electrons, e^-_{aq}, are produced as the main reactive species. In the presence of N_2O solvated electrons are converted into $OH^\cdot$ radicals in a fast reaction: $N_2O + e^-_{aq} + H_2O \rightarrow OH^\cdot + OH^- + N_2$. The radicals which subsequently react with solute then consist to 90% of $OH^\cdot$ and 10% of $H^\cdot$. N_2O was freed from traces of O_2 by passage through an oxysorb column (Messer-Grießheim). By using a mixture of N_2O/O_2 (4:1, v/v) the formation of $OH^\cdot$ by the above reaction of e^-_{aq} with N_2O is practically not inhibited but $H^\cdot$ atoms and carbon centered radicals (if not too short lived) are fully scavenged by O_2. γ-Radiolysis was carried out at 273 K at dose rates around 0.1 Gy s^{-1}, doses ranged from 65 to 300 Gy causing 2 to 8% conversion of the substrates which were 10^{-2} M. The radiation chemical yields are given in *G*-values which are defined as the number of molecules formed (or decomposed) per 100 eV of radiation energy absorbed. 1 Gy = 6.24×10^{15} eV g^{-1}.

Ammonia

After γ-irradiation solutions were set to pH > 12 and NH_3 was measured potentiometrically with a NH_3-sensitive electrode (Orion), calibrated with NH_4Cl solutions. NH_3 concentrations were found to be linear with dose while the unirradiated blanks were practically free of NH_3.

Measurements of radical decay rates

Time-resolved changes of d.c. conductivity (time resolution 1 μs) were measured after a 2.8 MeV electron pulse of 1 μs duration [15]. Inherent to the method is a signal component right after the pulse which is due to the radiation-induced formation of H^+ and OH^-. This signal component is pH- and dose-dependent. A further signal component is a reproducible dose-dependent noise signal. To account for these signals they were measured together using solutions of 4-hydroxyproline (which additionally gave rise to a very small but just detectable permanent conductivity increase) at the same concentration (10^{-2} M), pH (5.8) temperature, and doses as used with serine or threonine, respectively. With computer aid these signals were subtracted from those obtained with serine or threonine, respectively. The resulting curves were automatically analyzed by computer. First order rate constants thus obtained were extrapolated to zero dose.

All measurements were done at N_2O-saturation.

Results and Discussion

ESR spectroscopy

Serine

In acidic solutions up to pH 5.4 signals of the radicals **3a** or **4a** were observed. Small differences in the couplings observed as compared to published data [10–12] are attributed mainly to the lower temperatures used in our experiments. We found a pH dependence of the couplings which is most pronounced with the values for a^H_β, see Fig. 1. The half value of the changes is at pH 2.2. Therefore a pK_a of radical **3a** at 2.2 (at 276 K) is assumed. It is assigned to the COOH function of **3a** since the OH couplings are well resolved and protonation of the amino function in the above pH range appears granted. Our finding supports the earlier assumption [10, 11] that the pK_a's of the α-hydroxyalkyl radicals derived from serine or threonine (**3a** and **3b**) are not far apart from those of the amino acids, *cf.* Scheme 1. The pK_a of the OH function will be discussed later.

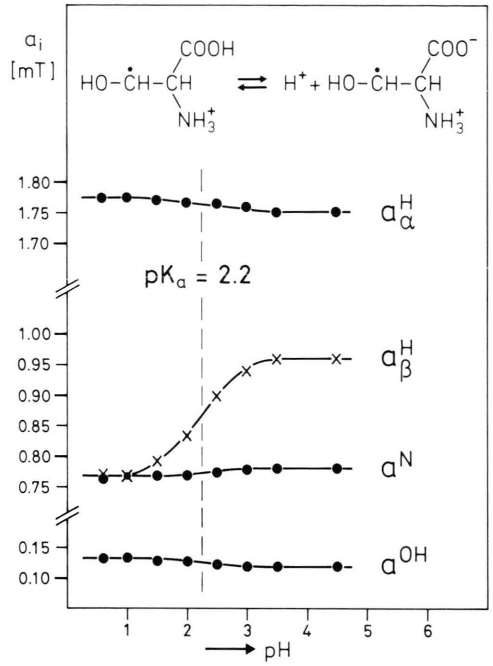
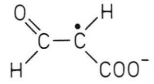


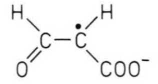
Fig. 1. Splittings a^H and a^N of the radicals HO–ĊH–CH(COOH)NH$_3^+$, **3a**, and HO–ĊH–CH(COO$^-$)NH$_3^+$, **4a**, in acid-base equilibrium as a function of pH, 276 K, 10^{-2} M serine.

Above pH 4 the signals of the zwitterionic radical **4a** decreased in intensity and they were no longer observed at pH > 5.4, but new signals grew in instead. These are assigned to the radical anion O=CH–ĊH–COO$^-$ (**7**) which is then formed by elimination of NH$_4^+$ from **4a** according to the overall reaction (5).

$$\underset{\mathbf{4a}}{\mathrm{HO{-}\dot{C}H{-}CH(COO^-)NH_3^+}} \rightarrow \mathrm{NH_4^+} + \underset{\mathbf{7}}{\mathrm{O{=}CH{-}\dot{C}H{-}COO^-}} \qquad (5)$$

The pH half value, the pH at which one half of the radicals **4a** undergoes decay into **7** and the other half undergoes bimolecular racical-radical termination, is estimated to 4.7 ± 0.1. The signal intensity of **7** began to decrease at pH ⩾ 8 and at pH 9 the signals were no longer observable. The reason for this decrease in alkaline solution will be discussed in context with γ-radiolysis experiments. The spectrum of **7** is asymmetric with respect to line heights and line shapes, a picture which may be expected with a mixture of two species with similar splittings but slightly different g-factors. Therefore a mixture of *E*- and *Z*-conformers, *E*-**7** and *Z*-**7**, is assumed*:


E-**7**;


Z-**7**

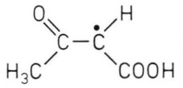
$a_\alpha^H = 1.76$ mT, $a_\beta^H = 0.7$ mT, $g = 2.0049$

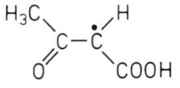
Threonine

Radical **3b** was observed only in rather acidic solutions. The ESR parameters at 276 K and pH 1 were: a_β(1H): 0.46 mT, a_β(3H): 2.10 mT, a(OH): ≈ 0 mT, a^N: 0.71 mT and $g = 2.0033$. The lines are unusually broad, ⩾ 0.03 mT, with half widths in the order of the expected OH splitting which is not resolved at our conditions. The splittings differ slightly from those reported earlier at 300 K [11].

Radical **3b** decreased in signal intensity already at pH ⩾ 0.8 and was no longer observable at pH 2.5. New signals grew in instead. The couplings proved to be pH dependent and were moreover asymmetric with regard to line heights and line profiles. Assignment is therefore and in view of the size of the couplings to *Z*- and *E*-conformers of the neutral radical CH$_3$–CO–ĊH–COOH (**8**) or of the anion radical CH$_3$–CO–ĊH–COO$^-$ (**9**), respectively, depending on the pH of observation. The pH dependence is depicted in Figure 2.

From the inflection points of the curves in Fig. 2 a pK$_a$ for radical **8** of 2.7 is derived. A pK$_a$ of similar size is expected for


E-**8**;


Z-**8**

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* There is an earlier assignment [16] of an ESR spectrum to radical **7**. That spectrum was obtained by γ-irradiation of propiolic acid at 77 K. But only the major coupling of 1.8 mT and a g factor of 2.005 could be measured with low accuracy. An assignment to the radical O=CH–ĊH–COOH [17] refers to ESR signals obtained upon photolysis of tartaric acid at 302 K. An a_H of 1.847 and a g factor of 2.00506 were measured there. In view of the high resolution of those spectra it is surprising that no small couplings could be seen. We therefore believe that this earlier assignment needs revision.

the radical $O{=}CH-\dot{C}H-COOH$ and therefore the assignment of the product radical observed with serine at pH > 4 was to the anion radical **7**.

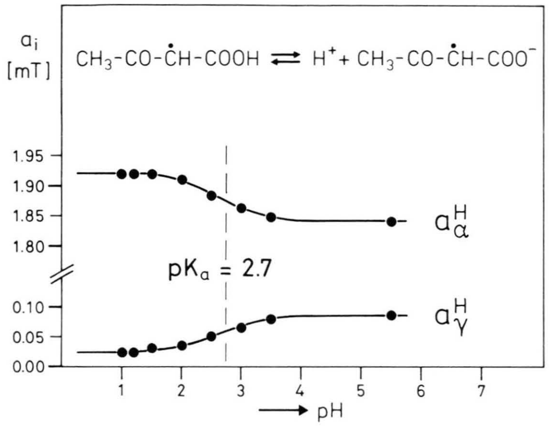

Fig. 2. Splittings a^H of the radicals $CH_3-CO-\dot{C}H-COOH$, *E*-**8** and *Z*-**8**, and $CH_3-CO-\dot{C}H-COO^-$, *E*-**9** and *Z*-**9**, in acid-base equilibrium as a function of pH, 276 K, 10^{-2} M threonine; g(average) = 2.005.

The gross spectral changes with pH clearly demonstrate the elimination of ammonia from an α-hydroxyalkyl radical of threonine. The observations may be described by the overall reaction (6).

$$\underset{\textbf{3b}}{CH_3-\dot{C}(OH)-CH(COOH)NH_3^+} \rightarrow$$
$$NH_4^+ + \underset{\textbf{8}}{CH_3-CO-\dot{C}H-COOH} \qquad (6)$$

The increase in signal intensity of radicals **8** was steepest between pH 1.5 and 2.0, that is around three pH units below the pH range of the steepest increase of radicals **7** from serine. From this large difference it became suspicious that the α-hydroxyalkyl radicals from serine and threonine decayed along different pathways since only small differences were expected should the radicals decay by the same reaction mechanism. We therefore checked the behaviour of the radicals **1a** and **1b** which are known to decay by the same mechanism, reaction (4), and which are otherwise the structural analogues of **4a** and **4b**.

2-Ammonioethanol and 1-ammonio-2-propanol

The known decay of radicals **1a** and **1b** [8] was confirmed, *cf.* reactions (4) and the Introduction. The splittings observed for **1a** at 276 K were: a^H_α = 1.83 mT, a^H_β = 1.15 mT, $a^H(OH)$ = 0.13 mT, a^N = 1.05 mT, and $a^H(NH_3)$ = 0.03 mT. The signals of **1b** were weak and broad. The pH half-values for the elimination of NH_4^+ from **1a** was 5.0 ± 0.1 and from **1b** 5.5 ± 0.1, the former value being determined from the decrease of the signal intensity of **1a** the latter determined from the increase of signal intensity of $\dot{C}H_2-CO-CH_3$.

Mechanism of ammonia elimination from the serine-derived radical

We can already conclude at this stage that the serine-derived zwitterionic radical **4a** decays in an analogous way as the radicals **1a** and **1b** derived from the ammonioalcohols. The observed stabilization by acid of **4a** against decay occurs in the pH range of 5.4 to 4.0. This number is well above the $pK_a(COOH)$ of radical **3a** (2.2) and well below the pK_a of the NH_3^+ group which is assumed to be > 9. Therefore, the stabilization by acid is mechanistically to be assigned to the protonation of the ketyl radical **5a** to yield **4a**, reactions (7), Scheme 3. The breakage of the C-N bond occurs at the stage of the ketyl radical. The overall reaction is reaction (5).

A quantitative treatment is based on further results which follow. That treatment will also exclude the possibility that the threonine-derived radical **4b** decays in analogy to the serine-derived radical **4a**.

$$\underset{\textbf{4a}}{HO-\dot{C}H-CH(COO^-)NH_3^+} \underset{(7r)}{\overset{(7f)}{\rightleftarrows}} H^+ + \underset{\textbf{5a}}{{}^-O-\dot{C}H-CH(COO^-)NH_3^+}$$
$$\underset{\textbf{5a}}{{}^-O-\dot{C}H-CH(COO^-)NH_3^+} \xrightarrow[(7d)]{} \underset{\textbf{7}}{O{=}CH-\dot{C}H-COO^-} + NH_3$$

Scheme 3. Mechanism of ammonia elimination from the serine-derived zwitterionic radical **4a** in aqueous solution.

Time-resolved measurements of electrical conductivity changes upon pulse radiolysis

Measurements with serine or threonine were done at pH 5.4 which number is close to the values of the isoelectric points of the amino acids (serine: 5.7, threonine: 5.6). In our measurements the zwitterionic amino acids behaved practically like electrically neutral substrates and what is measured is the formation of mobile ions, as is indicated *e.g.* by the gross reaction (5). The mobility of NH_4^+ is roughly $\frac{1}{5}$ of the mobility of H^+ (a species otherwise frequently encountered in such measurements) and the mobility of the radical anions **7** or **8** is estimated to be roughly $\frac{1}{10}$ of the H^+ mobility. Thus the net changes were expected (and found) to be relatively small. It is assumed that there is not much of a gross change in anionic mobility after radical-radical termination. Measurements were performed at rather low dose rates in order to permit the radicals to undergo first order decay reactions much faster than bimolecular radical termination. The size of $\Delta\varkappa$ measured was in accord with the assumption of G values for ion formation of 4 to 5. The build up of conductivity (after application of corrections mentioned in the Experimental) was of first order and the half lives derived were: 170 ± 10 μs with serine at 290.4 K and 26 ± 2 μs with threonine at 289.6 K. These observed half lives are assigned to the lifetimes with respect to ammonia elimination of the zwitterionic radicals **4a** and **4b**, respectively.

Yields of NH_3 after ^{60}Co-γ-irradiation

G-values of NH_3 were determined after ^{60}Co-γ-irradiation of aqueous solutions of serine or threonine at 273 K either at saturation with N_2O or with a mixture of N_2O/O_2, 4:1, v/v with certain variation in the pH of the solutions. The results are summarized in Table I.

Table I. *G*-values* of NH_3 as a function of pH, determined after ^{60}Co-γ-irradiation of aqueous solutions at 273 K of 10^{-2} M serine or threonine; for details see Experimental; accuracy ± 5%.

Amino acid	pH	*G*(NH_3) saturation with N_2O	with N_2O/O_2
serine	0	1.4	
	1	2.8	
	2.2	3.6	
	3.9	4.8	1.3
	6.2	4.9	
	7.0		1.3
	10		4.0
threonine	0	2.8	
	1	4.8	
	3	4.5	
	3.6	4.0	
	5	4.0	
	6	4.2	0.8
	7		0.8
	8	4.0	
	9		2.8
	11		4.0

* For definition see Experimental.

It is seen that with serine at $N_2$0-saturation the NH_3-yields begin to decrease with decreasing pH at pH 3.9 from 4.8 down to 1.4 at pH 0. With threonine such decrease is seen to begin only at pH 0. With threonine there is an increase in G(NH_3) from 4.0 at pH 3.6 to 4.5 at pH 3 and to 4.8 at pH 1 which is beyond the limits of error. Below pH 3 the solvated electrons from the radiation-induced ionization of water are increasingly scavenged by H^+-ions and no longer by N_2O. At pH 2 this leads to a composition of the reactive radicals from water of approximately 40% $OH^\cdot$ and 60% $H^\cdot$. The increase in G(NH_3) is then explained by a higher selectivity of the $H^\cdot$ atoms compared with the $OH^\cdot$ radicals in abstracting H from the carbon which carries the OH group. Such effect is also expected with serine at pH ≤ 3. There, however, this effect meets with the onset of stabilization by acid of radical **4a** against elimination of NH_3 as was discussed above. It is therefore difficult to exactly determine a pH-half-value for the elimination of NH_3 from radical **4a** under γ-irradiation. We have taken a value of 2.0, and we believe that this is approximately the pH value at which at our irradiation conditions radical **4a** eliminates NH_3 and undergoes bimolecular termination (without NH_3-formation) both at the same rate. Fortunately, the uncertainty of this value does not have too much of a bearing on the conclusions derived from this value (see further below).

The extent of formation of the α-hydroxyalkyl radicals

Recognition by ESR spectroscopy of a certain radical as a major or even as the only species does not necessarily mean that one is dealing with the main radical produced*. Information as to the extent of formation of radicals **4a** or **4b** by $OH^\cdot$ radical attack at serine or threonine in aqueous solution may be gained from the results presented in Table I. Taking a *G*-value for the sum of $OH^\cdot$ and $H^\cdot$ of 6.6 in the N_2O-saturated solutions $G(NH_3)$ values of 4.9 for serine and of 4.0 for threonine mean that roughly 75 or 60%, respectively, of the radicals produced from the substrates lead to NH_3 formation. This is the maximum percentage of radicals **4** since also other radicals in these systems might give rise to some NH_3.

As is inferred from the conductivity measurements the radicals **4** are long-lived enough to be fully scavenged by oxygen. This will lead to the corresponding peroxyl radicals
$HO-CH(OO^\cdot)-CH(COO^-)NH_3^+$ or
$HO-C(CH_3)(OO^\cdot)-CH(COO^-)NH_3^+$. These are expected to decay analogous to other α-hydroxyalkyl peroxyl radicals [19] mainly into $HO_2^\cdot$ and $O{=}CH-CH(COO^-)NH_3^+$ or
$CH_3-CO-CH(COO^-)NH_3^+$, respectively, but anyway mainly not to give rise to NH_3. As may be seen from Table I at saturation with N_2O/O_2 $G(NH_3)$-values are low at pH 7. At pH 10 or 11 the radicals $HOCH_2-\dot{C}H-NH_2$ or $HO-CH(CH_3)-\dot{C}H-NH_2$ are the main radicals formed [18]. The peroxyl radicals thereof are expected to give high yields of NH_3 along their pathways of degradation as is inferred from the behaviour of analogous radicals [20]. G-values of 4 found in the presence of N_2O/O_2 in alkaline solution are in accord with this expectation. In view of the low $G(NH_3)$-values in acidic solution (N_2O/O_2) it is then concluded that the main radicals in alkaline solution can only be minor** ones in acidic solution. Thus *formation of the radicals **4** and their decay dominates the radiation chemistry of aqueous serine or threonine in neutral to acidic solutions.*

Observability of the decay reactions as a function of radical lifetime

While at pH 3.9 radical **4a** is "stable" in our ESR experiments it has undergone complete decay during γ-radiolysis in the presence of N_2O (Table I, serine). This difference is explained by the different mean radical lifetimes in the two sets of experiments: around 0.3 milliseconds at our ESR conditions and around 60 milliseconds during γ-radiolysis with respect to bimolecular radical termination. For details see the Appendix. First order radical decay reactions with somewhat longer half lives than 300 μs will therefore escape our ESR spectroscopic detection. At pH 4.0 the decay of radical **4a** became just detectable and it is understood that on a ≈ 200-fold longer time scale of mean radical life this radical has completely decayed.

To explain the variance with the earlier ESR observations with serine and threonine [10–12] it is assumed that there the mean radical lifetime with respect to bimolecular radical termination was significantly shorter than in our experiments. In the earlier experiments the $OH^\cdot$ radicals were produced by use of the Ti^{3+}/H_2O_2 rapid mixing system where certainly much higher rates of $OH^\cdot$ radical generation – and

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* This *caveat* has found recent justification in amino acid radical chemistry: In aqueous alkaline solutions of a variety of amino acids, not comprising serine or threonine, so-called α-radicals of the amino acids, $R-\dot{C}(COO^-)NH_2$, *e.g.* $R = CH_3$ for alanine, have been observed by ESR spectroscopy as the only H-abstraction radicals [12] by use of Ti^{3+}/H_2O_2/EDTA systems for $OH^\cdot$ radical generation. The α-radicals are however not the main species formed upon $OH^\cdot$ attack on the amino acids. Only very recently it was reported [18] that in alkaline solutions of aliphatic amino acids, including serine and threonine, the main process following $OH^\cdot$ attack consists of decarboxylation and formation of α-amino radicals $R-\dot{C}H-NH_2$. These are strongly reducing species [18] and it is suggested that it is their fast reaction with H_2O_2 which prevents ESR spectroscopic detection in such systems. This will also explain our failure to observe any radicals from serine at pH > 9. To explain the selective appearance of the amino acid α-radicals the earlier investigators [12] discussed a) differences in the regio selectivity of the $OH^\cdot$ attack, α to the NH_2 and β to the NH_3^+ group, in accord with the electrophilic nature of the $OH^\cdot$ radical, and b) involvement of Ti^{3+}/EDTA/amino acid complexes which they observed in some instances.

** With respect to the assignment of the conductivity build up to the decay of radicals **4** the behaviour of the minor radicals with respect to ion formation and in particular proton formation needs consideration. Formation of the neutral α-amino radicals does not lead to gross ion formation, the radical $\dot{C}H_2-CHOH-CH(COO^-)NH_3^+$ is a non conducting zwitterion and the α-radicals of the amino acids which are born as
$CH_2OH-\dot{C}(COO^-)NH_3^+$ or
$CH_3-CHOH-\dot{C}(COO^-)NH_3^+$ may be assumed to exist at pH 5.4 as neutral species
$CH_2OH-\dot{C}(COOH)NH_2$ or
$CH_3-CHOH-\dot{C}(COOH)NH_2$. The latter assumption is based on analogy with the pK_a's of the glycin-derived radical $H_2N-\dot{C}H-COOH$ for which values of 6.6 (COOH) and ≤ 1 (NH_3^+) have been reported [21, 22].

hence bimolecular termination and hence shorter mean radical lifetimes – are achievable than in our photolytic flow system, so that at the earlier conditions the first order decay reactions were too slow for being detectable. Thus earlier, radical **4a** was observed at pH 6.5 at which pH we observed the decay product **7** only and radical **3b** was earlier observed at pH 2 where we could just detect it and saw mainly radical **8**. The variance with the earlier observation can not be adduced to differences in H_2O_2 concentration since this was the same.

pH effects on radical lifetimes

The zwitterionic radical **4a** from serine

The observed rate constant for the decay of radical **4a**, *cf.* Scheme 3, reactions (7f), (7r) and (7d), is given by Eqn. (I) [23],

$$k_{\text{observed}} = \frac{k_{7\text{f}} \times k_{7\text{d}}}{k_{7\text{d}} + [\text{H}^+] \times k_{7\text{r}}} \quad \text{(I)}$$

see also the Appendix. $k_{7\text{f}}$ is related to the $pK_a(OH)$ of **4a** through the equilibrium constant K_a for the equilibrium **4a** $\leftrightarrows$ **5a** + H^+ by $k_{7\text{f}} = K_a \times k_{7\text{r}}$. The rate constant for protonation of **5a** is $k_{7\text{r}}$. $[H^+]$ is the proton concentration, and $k_{7\text{d}}$ is the rate constant for ammonia elimination from the ketyl radical **5a**. It may be seen that for $k_{7\text{d}} \gg [H^+] \times k_{7\text{r}}$ the k_{obs} approaches $k_{7\text{f}}$, for $k_{7\text{d}} = [H^+] \times k_{7\text{r}}$ the k_{obs} is twice $k_{7\text{f}}$, and for $k_{7\text{d}} \ll [H^+] \times k_{7\text{r}}$ very substantial stabilization of radical **4a** is achievable.

We have obtained estimates for $k_{7\text{f}}$ and $k_{7\text{d}}$ by the following procedure: The stationary state is considered at which half of the radicals **4a** decays bimolecularly and the other half decays *via* **5a** by ammonia elimination. We have the experimentally determined pH half values for the ESR and γ-radiolysis conditions. A value of $k_{7\text{r}} = 4.5 \times 10^{10}\ \text{M}^{-1}\text{s}^{-1}$ is selected from protonation rate constants for analogous reactions [24]. Radical generation rates are derived from G-values and dose rates for γ-irradiation conditions and such rates were determined experimentally for the ESR conditions. A rate constant for bimolecular radical termination of $2 \times 10^9\ \text{M}^{-1}\text{s}^{-1}$ is taken. Steady state considerations then led us to two equations containing the unknowns $k_{7\text{f}}$ and $k_{7\text{d}}$ from which the values $k_{7\text{f}}(\text{calc}) = 2.90 \times 10^3\ \text{s}^{-1}$ and $k_{7\text{d}}(\text{calc}) = 1.95 \times 10^6\ \text{s}^{-1}$ are derived. For details see the Appendix. With these calculated values, with $k_{7\text{r}}$ as assumed and equation (I) a $k_{7\text{obs}}(\text{calc})$ at pH 5.8 of $k_{7\text{f}} \times 0.963$ is obtained. This means about 4% of stabilization by acid of radical **4a** at pH 5.8.

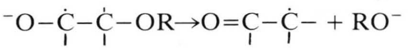
It is now assumed that the experimentally determined value as measured by time-resolved conductivity build-up on pulse radiolysis of $k_{\text{obs}}(\text{exp}) = 4.1 \times 10^3\ \text{s}^{-1}$ (from $\ln 2/\tau_{1/2}$, $\tau_{1/2} = 170 \times 10^{-6}$ s) at pH 5.8 is also about 4% lower than $k_{7\text{f}}(\text{exp})$, $k_{7\text{f}}(\text{exp}) = 4.25 \times 10^3\ \text{s}^{-1}$. This value of $k_{7\text{f}}(\text{exp})$ stands for a $pK_a(OH)$ value of radical **4a** of 7.0. With $k_{7\text{r}}$ as assumed and the values for $k_{\text{obs}}(\text{exp})$ and $k_{7\text{f}}(\text{exp})$ from equation (I) a value for $k_{7\text{d}}(\text{exp})$ of $2.4 \times 10^6\ \text{s}^{-1}$ is derived. The k(exp)-values refer to a temperature of 290 K. Summarizing:

$$\begin{array}{lll} \mathbf{4a} \rightleftarrows \mathbf{5a} + H^+ & \mathbf{5a} \rightarrow \mathbf{7} + NH_3 & (7\text{d}) \\ pK_a(OH) = 7.0 & k_{7\text{d}} = 2.4 \times 10^6\ \text{s}^{-1} & \\ & \text{at 290 K} & \end{array}$$

$k_{7\text{d}}$ is about the same size as the rate constants of the decay of related ketyl radicals [25], reactions (8) and (9).

$${}^-O-\dot{C}R_1-CHR_2-OH \rightarrow O=CR_1-\dot{C}HR_2 + OH^- \quad (8)$$

(R_1, R_2 = H, CH_3, CH_2OH;
$3.1 + 10^6\ \text{s}^{-1} \leqslant k \leqslant 8.1 \times 10^6\ \text{s}^{-1}$), or



$${}^-O-\underset{|}{\dot{C}}-\underset{|}{\overset{|}{C}}-OR \rightarrow O=\underset{|}{C}-\underset{|}{\dot{C}}- + RO^- \quad (9)$$

(R = alkyl; $k > 5 \times 10^5\ \text{s}^{-1}$)

There is apparently little change in the rate constants of decay of the ketyl radicals on alkyl substitution or with the nature of the leaving group.

The cationic radical **3a** from serine

The COOH group in **3a** is more electron attracting than the COO^- group in **4a**. It is therefore expected that the deprotonation of the OH group of **3a** is faster than in the case of **4a** and that the elimination of ammonia from the ketyl radical **6a** (Scheme 2) is slower than from the ketyl radical **5a**. While the first effect alone should lead to a larger k_{obs} for the decay of **3a** as compared with k_{obs} for **4a** the second effect will lead to stabilization of **3a** by acid at somewhat higher pH values than with **4a**. Since the pH range of existence of **3a** is lower than for **4a** the stability of **3a** is granted through the efficient reprotonation of the ketyl radical **6a**.

The radicals **1** from the ammonioalcohols

A certain increase of the $pK_a(OH)$ is expected on going from radical **1a** to radical **1b** – on C-methylation at the radical carbon – as may be inferred from a comparison of known $pK_a(OH)$ values of α-hydroxyalkyl radicals: $HO-\dot{C}H_2$ (10.7), $HO-\dot{C}H-CH_3$ (11.6), and $HO-\dot{C}(CH_3)_2$ (12.2) [7, 26], or $HO-\dot{C}H-COOH$ (8.8) and $HO-\dot{C}(CH_3)-COOH$ (9.8) [27], or $HO-\dot{C}H-CONH_2$ (5.5) and $HO-\dot{C}(CH_3)-CONH_2$ (6.5) [28]. Such an effect alone would lead to a slower decay of **1b** as compared with **1a**.

On the other hand it is known that C-methylation in a ketyl radical leads to a somewhat faster decay: k_8 for $^-O-\dot{C}H-CH_2-OH$ is $3.1 \times 10^6\ s^{-1}$ and $5.3 \times 10^6\ s^{-1}$ for $^-O-\dot{C}(CH_3)-CH_2-OH$ [24], see reaction (8). Assuming analogy this would lead to a faster decay of the methylated species. With radical **1b** the former effect seems to be somewhat more important than the latter.

The zwitterionic radical 4b from threonine

If the mechanism of decay of the serine-derived radical **4a** and the threonine-derived radical **4b** were the same a slightly larger pH half-value with **4b** compared with **4a** was to be expected, *viz.* around 0.5 units as was found on passing from **1a** to **1b**. The pH half value found by ESR spectroscopy was around three pH units lower with **4b** than with **4a**. This indicates a profound difference in the mechanisms of decay of the two radicals. There are three reasons to assume that this is true:

a) Considering the same mechanism of decay for **4a** and **4b** the ketyl radical **5b** should decay somewhat faster than the ketyl radical **5a** with the consequence that at pH 5.4 – where around 10 per cent stabilization by acid was calculated for k_{obs}(exp.) for **4a** – even less stabilization at this pH is expected for **4b**. Hence the practically non-inhibited decay of **4b** should be measured by $\Delta\varkappa$ changes. This measured decay rate should correspond to the deprotonation step of the OH group in **4b** which in this instance must be rate determining. Assuming the $pK_a(OH)$ of **4b** to be 0.3 to 0.7 units larger than of **4a** the k_{obs}(exp.) of **4b** should be two to five times smaller than with **4a** but observed was a 6.5 times larger value.

b) Another consequence of a slower and rate determining decay of **4b** would have been the observability of this species in the ESR spectra already at pH 5.4. At this pH radical **4a** was just detectable, but a radical with twice its half-life would not have escaped detection as is inferred from the good observability of **4a** at pH 4.7 where its half-life was approximately twice as long than at pH 5.4. However, nobody has so far presented an ESR spectrum of **4b**. And the fact of the matter is, as follows from the measured rate of decay of **4b**, that this radical is too short-lived for ESR observation at our conditions.

c) Disregarding for a moment the above considerations and facts and supposing the measured half-life of **4b** (≈ 26 μs) at pH 5.8 corresponds to the rate determining deprotonation at OH and assuming further that the ketyl radical **5b** eliminates NH_3 three times faster than **5a**, then stabilization by acid of **4b** should render this radical ESR-observable in an accessable pH range, *viz.* at pH 4 or slightly below. But this was also not the case. One might infer that **4b** is invisible in the ESR spectra *e.g.* because of line-broadening. But proof of its decay is the observation of the decay product, radical **8** or of its anion $CH_3-CO-\dot{C}H-COO^-$, **9**, depending on pH. Radical **8** appeared in the spectra at pH ≥ 0.8, was fully developed at pH 2, where **3b** had vanished, and **8** was only replaced by **9** at higher pH values. *There is no indication for stabilization by acid of the zwitterionic radical **4b**. Radical **4b** must decay in a pH-independent step. The stabilization by acid observed at pH < 2 is gained by the cationic radical **3b**.*

The mechanism of decay of the threonine derived α-hydroxyalkyl radicals

The mechanism of decay is given in Scheme 4.

$$\underset{\mathbf{3b}}{HO-\dot{C}(CH_3)-CH(COOH)NH_3^+} \underset{(10r)}{\overset{(10f)}{\rightleftarrows}}$$

$$\underset{\mathbf{4b}}{HO-\dot{C}(CH_3)-CH(COO^-)NH_3^+} + H^+ \quad (10)$$

$$\mathbf{4b} \rightarrow \underset{\mathbf{10}}{HO-\overset{+}{C}(CH_3)-\dot{C}H-COO^-} + NH_3 \quad (11)$$

$$\mathbf{10} \rightarrow H^+ + \underset{\mathbf{9}}{CH_3-CO-\dot{C}H-COO^-} \quad (12)$$

or:

$$\mathbf{4b} \rightarrow H^+ + \mathbf{9} + NH_3 \quad (13)$$

Scheme 4. Mechanism of decay of the threonine derived α-hydroxyalkyl radicals **3b** and **4b**.

The pH dependence of the ESR spectra is governed by the equilibrium, reactions (10f) and (10r).

Ammonia elimination occurs from the zwitterionic radical **4b** (in the serine case from the ketyl radical **5a**). In the absence of stabilization by acid C-N bond breakage is rate determining (H-O bond breakage in the serine case). The timing of the C-N and H-O bond breakage may be consecutive as indicated by reactions (11) and (12) where a new zwitterionic radical **10** is the intermediate. **10** may be regarded as protonated ketone with a probably negative pK_a value and hence be of very short life with respect to deprotonation, reaction (12), in weakly acidic solutions. Alternatively, deprotonation may set in after C-N bond breakage has begun but before this is finished. In that case NH_3 elimination may be better describes by reaction (13) where there is no intermediate but a transition state which differs somewhat from the transition state of reaction (11). We are facing the same mechanistic problems in reactions (1) mentioned at first in the Introduction.

The rate constant measured by conductivity buildup of threonine solutions on pulse radiolysis, $k = 0.69/26\ \mu s = 2.7 \times 10^4\ s^{-1}$ is then assigned to either reaction (11) or (13).

Factors determining the selection of a mechanism of heterolytic decay in aqueous solution of an α-hydroxyalkyl radical which carries a leaving group in β-position

We shall now discuss some of the factors which determine whether a given α-hydroxyalkyl radical cleaves its leaving group heterolytically at the stage of the α-hydroxyalkyl radical, like the threonine-derived radical **4b** – we shall name this a *type-A* cleavage, mechanism or reaction – or, whether heterolytic cleavage* occurs at the stage of the ketyl radical, as was shown for the serine-derived radical **4a** or was known for the radicals **1** – we shall name this a *type-B* cleavage, reaction or mechanism.

An attempt is made to classify some known reactions accordingly.

For a *type-A* reaction the relative electrophilicity of the organic skeleton and the leaving group propensity, or perhaps better the nucleofugacity of the leaving group will be of importance, for a *type-B* decay reaction also the $pK_a(OH)$ becomes an important factor.

α-Hydroxyalkyl radicals **11** are considered and compared with the analogous α-methoxyalkyl radicals **12** which also undergo heterolytic cleavage reactions in aqueous solution, reaction (14); X = leaving group. There, short lived (1–100 ns) and scavengeable (by HPO_4^{--}) radical cations are intermediates [31]. Analogy is expected with the *type-A* cleavage of radicals **11** as to the effect of substituents R′ and R″ or of the leaving group X on the rate of cleavage.

$$\underset{\mathbf{11}}{HO-\dot{C}R'-CHR''-X} \qquad \underset{\mathbf{12}}{CH_3O-\dot{C}R'-CHR''-X}$$

$$\mathbf{12} \xrightarrow{H_2O} CH_3O-\overset{+}{C}R'-\dot{C}HR'' + X^- \longrightarrow$$

$$CH_3O-CR'(OH)-\dot{C}HR'',$$
$$CH_3O-\dot{C}R'-CHR''OH + H^+ \qquad (14)$$

a) Electrophilicity of the organic skeleton

It is the +M effect of the OH group in α-position to the radical site in radicals **11**, or of the CH_3O group in radicals **12**, which contributes largest to the general ease of the heterolytic decay reactions [29, 30]. Replacing now the OH group by a O^- group – passing to the ketyl radicals – this must lead to a substantial decrease in electrophilicity of the organic frame and rate acceleration to an extent that even "bad" leaving groups are cleaved, in accord with observation. With radicals **12**, $X = HPO_4^-$; R′, R″ = H, upon substitution of H by CH_3 at the α- or at the β-position an increase of the cleavage rate by a factor of ≈ 10^5 was found [31], which in tendency but not necessarily in size may also be expected in the *type-A* decay of radicals **11**. Large methyl group effects on reaction rates are usually found once in the transition states cationic partial structures are stabilized (such effects being much less pronounced for the stabilization of radical partial structures). It is basically such methyl group effect on an incipient radical-cationic structure of a transition state that brings about the change-over in reaction mechanism from a *type-B* decay of radical **4a** (through the ketyl **5a**) to the *type-A* cleavage of radical **4b**.

The methyl group effect was not strong enough to lead to a change-over in mechanism on going from radical **1a** to **1b**. The mechanistic change is brought about by the introduction of a COO^- group on going from **1b** to **4b**, however not by the introduction of a COOH group on going from **1b** to **3b**.

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* The following discussion will not include the homolytic cleavage reactions of β-positioned groups which might occasionally interfere with the heterolytic ones.

The situation may be understood with the aid of the TAFT equation, Eqn. (II),

$$\log k = \text{const.} - (\varrho^* \times \sigma^*) \qquad \text{(II)}$$

where now k is the rate constant of a *type-A* reaction, ϱ^* is the susceptibility of such reaction, here the elimination of NH_3, towards substituent effects on reaction rate constants, and σ^* are "polar" substituent constants, for a list of such constants see ref. [32]. It is $\triangle \log k = \varrho^* \times \triangle \sigma^*$. With the σ^*-values for COOH (2.08) > H (0.49) > CH_3 (0.0) > COO^- (−1.06) it follows that the electrophilicity of the organic skeleton decreases in the above sequence. The expected sequence of rate constants for a *type-A* decay for the radicals dealt with in this paper is then **3a** < **3b** < **1a** < **1b** < **4a** < **4b**. Only radical **4b** actually decays by the *type-A* mechanism. For the other radicals the decrease in electrophilicity prevents it on our time scale of mean radical lives*.

b) Nucleofugacity of the leaving group

Within a given set of radicals the mechanistic border between species decaying according *type-A* or *type-B* mechanism also depends on the nature of the leaving group.

With radicals **12**, R′, R″ = H and X_1 = $OCOCH_3$, X_2 = $OCOCH_2OCH_3$ or X_3 = $OCOCH_2Cl$ the rate of decay was of the sequence (in terms of the leaving group) $X_1 < X_2 < X_3$. The same sequence is expected for the *type-A* decay of analogous radicals **11**. In these instances there is a rate increase with increasing electrophilicity *and* increasing nucleofugacity of the leaving group. Electrophilicity and nucleofugacity however do not always go parallel which may be demonstrated with the sequence of rate constants of *type-A* cleavage of the radicals** $HO-\dot{C}H-CH_2-NH_3^+$, **1a**, < $HO-\dot{C}H-CH_2-OCOCH_3$, **13**, < $HO-\dot{C}H-CH_2-Cl$, **14**. Here, while the electrophilicity of the skeleton is held constant, it is the nucleofugacity of the leaving group which determines the sequence. As a measure of the nucleofugacity the $pK_a(HX)$ of the conjugate acid of the leaving group may be taken: HCl (negative pK_a), $HOCOCH_3$ (4.7), NH_4^+ (9.3), and a high pK_a corresponds to a low nucleofugacity. The electrophilicity of the leaving groups in **1a**, **13** and **14** is adequately described by σ^*-values: NH_3^+ (3.76), Cl (2.96), $OCOCH_3$ (2.56), and it is seen that this sequence does not correspond to the sequence above.

The mechanistic border between the *type-B* and the *type-A* decay on the coordinate of nucleofugacity is between radicals **1a** and **13.**

c) The $pK_a(OH)$ of the α-hydroxyalkyl radical

It is the above sequence of σ^*-values that determines the sequence of the $pK_a(OH)$-values of the radicals **1a** < **13** < **14**, large σ^*-values corresponding to low pK_a-values. And it is the decrease in $pK_a(OH)$ of radical **1a** below a certain value which is one condition to render the *type-B* decay observable to ESR spectroscopy, the other condition being a fast enough decay of the corresponding ketyl radical. With a very electrophilic skeleton this observability is no longer granted, and the radical $CF_3-\dot{C}(O^-)-CF_3$, **15**, may serve as an example. Because of the very electrophilic skeleton the $pK_a(OH)$ of the radical $CF_3-\dot{C}(OH)-CF_3$, **16**, is as low as 1.76 [26], but from the same reason the rate of elimination from the ketyl radical **15** is that low that it was well observable by ESR [26]. We found that F^--elimination takes place upon $OH^{\cdot}$ radical attack on hexafluoro-propan-2-ol in aqueous solution at ^{60}Co-γ-irradiation, and the result is another example of the influence of the mean radical life on the observability of a radical decay.

Since now radical **16** decays by a *type-B* mechanism we are able to point out another borderline on the coordinate of the electrophilicity of the skeleton, *viz.* one between radical **16** and the radical $HO-\dot{C}H-CH_2-F$, **17**, which latter decays pretty fast [2] and probably does so by a *type-A* mechanism, see further below. To narrow the large gap in skeletal electrophilicity between radicals **16** and **17** and in order to test the wider applicability of the principles outlined in the preceeding chapters we briefly investigated the behaviour of the radical $HO-\dot{C}H-CF_3$, **18**, which we generated from 2,2,2-trifluoroethanol by H-abstraction under otherwise the same condi-

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* This treatment is considered a first approximation. It is assumed that with the appearance of more kinetic data also other factors not adequately represented by σ^*-values may become recognizable, as there are mesomeric and steric effects on transition state stabilization and effects thereon from charge concentration or charge dissipation, respectively.

** k(**13**) = $5 \times 10^5\ s^{-1}$ [2], k(**14**) must be higher than k(**13**) since scavenging by O_2 was practically not possible k(**1a**) for a *type-A* cleavage must be below $10^3\ s^{-1}$ because in this instance *type-B* decay became faster.

tions as radicals **4a** or **4b**. Radical **18** was expected to possess a pK_a half way in between that of $HO-\dot{C}H-CH_3$ and that of **16**, *viz.* around 6, and the corresponding ketyl radical was expected to decay faster than **15**, so that both conditions for the observability of a *type-B* decay were possibly fulfilled. The experiments confirmed this: the radical **18** was well observable up to pH 3.8: $a_\alpha^H = 1.855$ mT, $a_\beta(3F) = 3.170$ mT, $a(OH) = 0.15$ mT, $g = 2.00323$, half-line-width ≈ 0.005 mT, at 277K. The signal intensity began to decrease at pH 3.8 and this radical was no longer observable at pH 7.1. From pH 7.1 – 10 the radical $\dot{C}F_2-CH=O$, **19**, appeared instead: $a_\alpha(F_1) = 4.7$ mT, $a_\alpha(F_2) = 4.3$ mT, $a_\beta^H = 0.35$ mT, $g = 2.00553$, half-line-width ≈ 0.15 mT. The mechanistic change-over from *type-B* to *type-A* mechanism is then to be sought between the radicals **18** and $HO-\dot{C}H-CHF_2$ or between the latter and **17**.

The $pK_a(OH)$-value of 7.0 determined in this work for radical **4a** permits an estimate of the pK_a-value of radical **14**: the sum of the σ^*-values of the COO^- and the NH_3^+ group amounts to 2.7, the value for Cl being 2.96 is only slightly larger. From this it is assumed that the $pK_a(OH)$* for radical **14** is slightly below 7, and since **14** decays with a rate constant $> 10^6$ s^{-1} and since stabilization by acid was not seen, radical **14** is assumed to decay by the *type-A* mechanism. The same set of arguments applies to radicals **13** and **17**. Quite generally speaking the *type-B* mechanism – besides with radicals with a rather electrophilic skeleton – requires electron attracting substituents which in the same time are "bad" leaving groups. Possible candidates include the group of the nucleobases and other nitrogen heterocycles and research along these lines will be continued.

Appendix

Stationarity is assumed for our ESR spectroscopic as well as ^{60}Co-γ-irradiation conditions concerning radical **4a**. At pH half value the rate of decay of **4a** equals the rate of formation of **7** and equals the rate of bimolecular termination of **4a**.

Then it is

$$k_{7f}\,[\mathbf{4a}]\,k_{7d}\,[\mathbf{5a}]/(k_{7d}\,[\mathbf{5a}] + k_{7r}\,[H^+]\,[\mathbf{5a}]) = k_{bim}([\mathbf{4a}]^2 + [\mathbf{7}]\,[\mathbf{4a}] + [oth]\,[\mathbf{4a}]),\text{ or}$$

$$k_{7f}k_{7d}/(k_{7d} + k_{7r}[H^+]) = k_{bim}([\mathbf{4a}] + [\mathbf{7}] + [oth]) = k_{bim}[R^\cdot]$$

Concentrations of radicals are given in brackets, [oth] is the concentration of all other radicals than **4a** and **7** derived from serine, k_{bim} is the average bimolecular termination rate constant of the radicals for which a value of 2×10^9 $M^{-1}s^{-1}$ is taken. $[R^\cdot]$ is the concentration of all serine derived radicals. The other symbols have been explained in the text. For k_{7r} a value of 4.5×10^{10} $M^{-1}s^{-1}$ [24] is taken.

The production rate of all serine derived radicals, $R^\cdot$, at γ-irradiation (N_2O), taking a *G*-value of 6.6, at 0.11 Gy s^{-1} is 7.2×10^{-8} M s^{-1} which at stationarity equals $k_{bim}\,[R^\cdot]^2$, so that $[R^\cdot] = 6.2 \times 10^{-9}$ M.

The production rate of $OH^\cdot$ radicals in our ESR photolytic flow system was determined by the measurement of the rate of Cl^- formation which follows $OH^\cdot$ attack on 2-chloroethanol in 10^{-2} M aqueous solution at 276 K. For the reaction $OH^\cdot + HO-CH_2-CH_2-Cl \rightarrow H_2O + HO-\dot{C}H-CH_2-Cl \rightarrow Cl^- + O=CH-\dot{C}H_2$ [1, 2] a *G*-value of 5.4 ± 0.15 was determined, so that $\approx 80\%$ of the $OH^\cdot$ radicals produced give Cl^-. A value of 1.0×10^{-6} M for $[R^\cdot]$ was thus determined for our ESR spectroscopic conditions.

Using now the observed half values of pH of 4.7 for the ESR and of 2.0 for the γ-irradiation conditions we arrive at two equations containing the unknowns k_{7f} and k_{7d}:

ESR: $k_{7f}k_{7d}/(k_{7d} + 9 \times 10^5) = 2000$
γ-irradiation: $k_{7f}k_{7d}/(k_{7d} + 4.5 \times 10^8) = 12.4$

The result is:

$k_{7f} = 2.9 \times 10^3$ s^{-1} and $k_{7d} = 1.95 \times 10^6$ s^{-1}.

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* Although there is already a correlation of σ^*-values with the $pK_a(OH)$-values of radicals "$\dot{R}-OH$" [33] – which does not include our labile radicals – that correlation shows too much scatter to be useful in estimating radical pK_a's for **13**, **14** or **17** or any of the other labile species. It was assumed [33] that the scatter was due to mesomeric effects of α-substituents not well comprised by σ^*-values.

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