

Model Reactions for the Degradation of DNA-4' Radicals in Aqueous Solution. Fast Hydrolysis of α -Alkoxyalkyl Radicals with a Leaving Group in β -Position Followed by Radical Rearrangement and Elimination Reactions

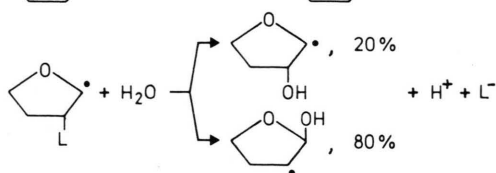
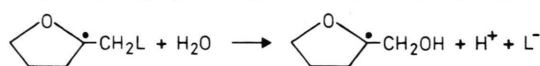
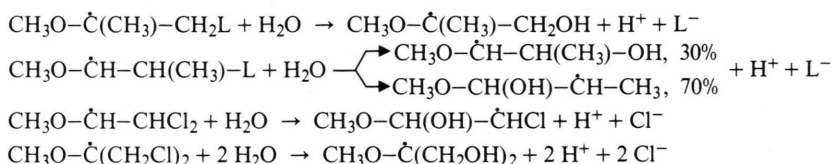
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Z. Naturforsch. **37c**, 1205–1227 (1982); received July 27, 1982

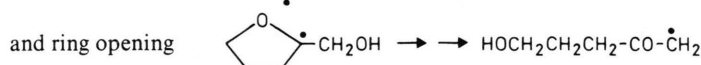
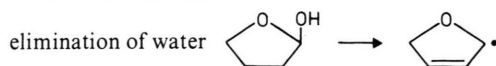
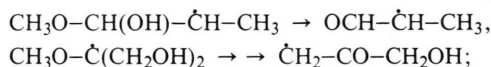
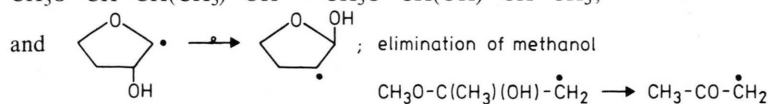
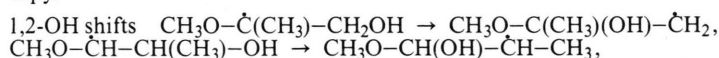
Radical Hydrolysis, Radical Rearrangement, α -Alkoxy- β -chloro(or acetoxy)alkyl Radicals, α -Alkoxy- β -hydroxyalkyl Radicals, β -Alkoxy- β -hydroxyalkyl Radicals

α -Alkoxyalkyl radicals with a leaving group $L = \text{Cl}$ or OCOCH_3 in β -position are produced by H-abstraction from the corresponding saturated substrates by $\cdot\text{OH}$, $\text{SO}_4^{\cdot-}$ or $(\text{CH}_3)_3\text{CO}\cdot$ radicals. From ESR spectroscopic observations it is concluded that in aqueous solution at pH 5–9 the following fast hydrolysis reactions take place:



The rate constants of these reactions and for the hydrolysis of $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2\text{Cl}$ are $k \geq 10^6 \text{ s}^{-1}$, whereas the rate constant for $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2\text{OCOCH}_3$ was determined to be $\approx 2 \times 10^3 \text{ s}^{-1}$ at room temperature. The radicals with $L = \text{Cl}$ cannot be scavenged by O_2 which fact leads to a value of $k \geq 2 \times 10^7 \text{ s}^{-1}$. α -Alkoxyalkene radical cations are assumed as intermediates in the hydrolysis reactions. The radicals with $L = \text{OCOCH}_3$ and the radical $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2\text{Cl}$ are observable in acetone solution ESR spectroscopically.

In aqueous solution at pH below 3 proton catalyzed reactions are observed by ESR spectroscopy:



Sonderdruckanforderungen an Prof. Dr. D. Schulte-Frohlinde.

0341-0382/82/1100-1205 \$ 01.30/0



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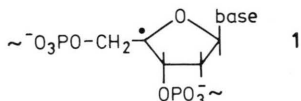
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Radicals resulting from H-abstraction at the CH₃O-groups of the substrates or at the 5-positions of the cyclic ethers are also observed. The ESR parameters and the pH-ranges of existence of the above radicals are given. Support of the reported reactions comes from quantitative analysis of stable products such as H⁺, Cl⁻ or CH₃OH after ⁶⁰Co-γ-irradiation of N₂O saturated aqueous solutions of the substrates.

The behaviour of the radicals is used as a model to describe a modified version of the degradation of DNA-4' radicals in aqueous solution in the absence of oxygen.

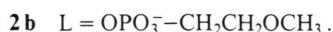
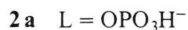
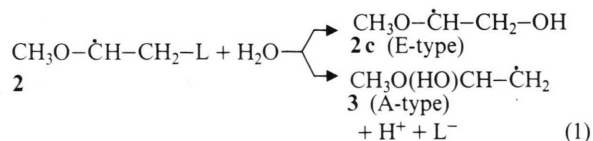
Introduction

The chemical pathways of radical induced strand break formation of DNA in aqueous solution are a matter of continuing interest. The results presently available show that the generation of radicals at the sugar moiety of DNA is responsible for the breakage of the strand [1]. Among these radicals the DNA-4' radicals, **1**, are particularly prone to lead to strand breaking reactions.



It is now well established [1] that in these reactions hydrolytic cleavage of the alkylated phosphate groups in the β-position to the radical site is involved. The hydrolysis reactions are rationalized in terms of a stepwise process with the primary formation of alkoxyalkene radical cations which subsequently react with water [1].

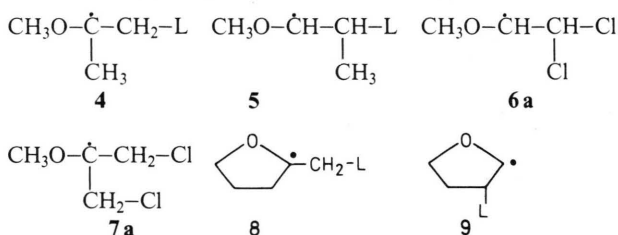
In a first paper [2] on rather simple models for **1** we reported on hydrolysis reactions of 1-methoxyethyl radicals, CH₃O-ĊH-CH₂-L, **2**, carrying as leaving groups (L) differently protonated or alkylated phosphate groups in the 2-position, *e.g.* **2a**, L = OPO₃H⁻, or **2b**, L = OPO₃⁻-CH₂CH₂OCH₃. According to reaction (1) two radicals, **2c** and **3**, are formed on hydrolysis which as type will be referred to in the following as ether type (E-type) or alkyl type (A-type) radicals respectively, *cf.* reaction (1).



At pH ≤ 1.8 the E-type radical **2c** is converted into the A-type radical **3** [3].

In this communication we report on the hydrolytic behaviour of some radicals which in compari-

son with **2** are closer in structure to **1**, *viz.* **4** to **9** with leaving groups L = Cl or OCOCH₃*.



4a, 5a, 8a, 9a: L = Cl; **4b, 5b, 8b, 9b:** L = OCOCH₃

It is shown that these radicals undergo fast hydrolysis reactions yielding E- and A-type radicals, which in acidic solutions undergo rearrangement and elimination reactions. On the basis of these findings a modified mechanism of degradation of radicals **1** in aqueous solution is advanced.

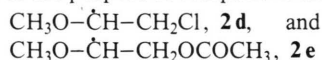
Results

Pulse radiolysis

The radicals were generated within the pulse length of one microsecond by H-abstraction with radiolytically produced OH radicals from corresponding saturated substrates, see Experimental. Using time-resolved electrical conductivity measurements as probe [4] with each of the substrates an increase of conductivity was found. This increase was smaller than expected for a 1 : 1 ratio between reacting OH radicals and protons produced. It is therefore concluded that not all radicals formed by H-abstraction decayed by hydrolysis with formation of acid.

With one exception the half lives of the decaying species were less than 1 microsecond, the time resolution of our conductivity set-up. In these cases the

* For the purpose of comparison the radicals



which also carry these leaving groups are included in this study.

formation of acid upon pulse radiolysis was practically complete within the pulse duration of 1 microsecond. The exception was found with $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OCOCH}_3$ as substrate where a first order build-up of conductivity with a half life of about 350 microseconds at 293 ± 3 K was observed.

ESR spectroscopy

As expected from the foregoing some of the H-abstraction radicals undergo rapid hydrolysis and others are inert to hydrolysis and do not change their structure during their radical life over the whole pH-range employed. Some of these latter radicals were detected and identified by ESR spectroscopy. They served as useful internal standards especially to monitor the changes in pH which the





successors of the short-lived H-abstraction radicals underwent. The ESR parameters of the "inert" radicals are given in Table I.

Radicals observed at pH 5–9

Besides the "inert" radicals (Table I) A-type and E-type radicals are observed, see Table II and Figs. 1–4. The A- and E-type radicals are considered to be the hydrolysis products of the model radicals **2d**, **4a** to **9a**, **4b**, **5b**, **8b**, and **9b** which are expected upon hydrogen abstraction from corresponding substrates but which were not observed by ESR spectroscopy in aqueous solutions.

The percentage of A- and E-type radicals (the product pattern) observed at pH 5–9 is given in Scheme 1. Reaction (2) was already known [2, 5] ex-

Table I. ESR parameters of radicals formed in aqueous solution at 276 K by H-abstraction from selected ethers at the methoxyl group or at the 5-position of tetrahydrofuran derivatives respectively. Hyperfine splittings in mT, number of protons larger than 1 in parentheses

Radical	a_{α}^{H}	a_{β}^{H}	a_{γ}^{H}	a_{δ}^{H}	Other splittings	g Factor
$\dot{\text{C}}\text{H}_2\text{OCH}_2\text{CH}_2\text{—Cl}$	1.728 (2) ^a	—	0.195 (2)	0.016 (2)	³⁵ Cl 0.015	2.00318
$\dot{\text{C}}\text{H}_2\text{OCH}_2\text{CH}_2\text{—OPO}_3\text{H}_2^{\text{c}}$	1.718 (2) ^a	—	0.196 (2)	0.016 (2)	P 0.016	2.00320
$\dot{\text{C}}\text{H}_2\text{OCH}_2\text{CH}_2\text{—OCOCH}_3$	1.726 (2) ^a	—	0.200 (2)	0.016 (2)	—	2.00321
$\dot{\text{C}}\text{H}_2\text{OCH}(\text{CH}_3)\text{CH}_2\text{—OCOCH}_3$	1.71 (2) ^a	—	0.127	0.015 (4)	—	2.00325
$\dot{\text{C}}\text{H}_2\text{OCH}_2\text{CH}(\text{CH}_3)\text{—OCOCH}_3$	1.72 (2) ^a	—	0.210 0.175	0.00	ϵ' 0.007 (3)	2.00324
$\dot{\text{C}}\text{H}_2\text{O—CH}(\text{CH}_2\text{Cl})_2$	1.745 (2) ^a	—	0.110	0.015 (4)	³⁵ Cl 0.012 ³⁷ Cl 0.010	2.00325
$\dot{\text{C}}\text{H}_2\text{O—CH}_2\text{—CHCl}_2$	1.74 (2)	—	0.157 (2)	0.019	³⁵ Cl 0.019 ³⁷ Cl 0.016	2.00317
	1.280 ^b	2.545 3.190	0.205	—	γ -H 0.072 (2)	2.00321
	1.28	2.54 3.19	0.215	—	γ -H 0.075 (2) ³⁵ Cl 0.01	2.0032
	1.315 ^c	1.99 3.77	0.330 0.032	—	γ -H 0.09	2.0032
	1.32	1.87 ^d 3.73 ^d	0.36 0.04	—	γ -H 0.09 ³⁵ Cl 0.003	2.0032

^a Broadening of the central line of the CH_2 triplet indicates non-equivalence of the two H's, cf. Figs. 5A, 6 and ref. [6].

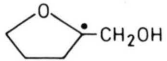
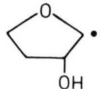
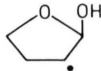
^b $d|a|/dT = -0.78 \mu\text{T/K}$ at 213 K... 264 K in acetone.

^c $d|a|/dT = -0.40 \mu\text{T/K}$ at 223 K... 261 K in acetone.

^d Error ± 0.05 mT due to multiple line overlap.

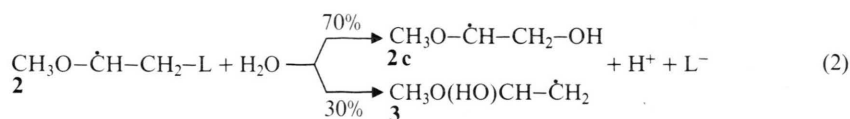
^e Data added for comparison.

Table II. ESR parameters and pH range of observation of radicals formed upon hydrolysis of α -alkoxyalkyl radicals carrying a leaving group in β -position in aqueous solution at 276 K. Number of protons larger than 1 in parentheses, hyperfine splittings in mT.

Hydrolyzing radicals	Radical observed	pH range	a_x^H	a_β^H	a_γ^H	a_δ^H	a^{OH}	g Factor	References
4a, 4b	$\text{CH}_3\text{O}-\dot{\text{C}}(\text{CH}_3)-\text{CH}_2\text{OH}$ 4c	3 – 11	—	$\begin{cases} 0.785 (2)^a \\ 2.166 (3) \end{cases}$	0.185 (3)	—	0.013	2.00302	*
4a, 4b	$\text{CH}_3\text{O}-\text{COH}(\text{CH}_3)-\dot{\text{C}}\text{H}_2$ 14	2 – 3.3	2.25 (2)	—	—	0.225 (3)	—	2.00246	*
4a, 4b	$\text{CH}_3-\text{CO}-\dot{\text{C}}\text{H}_2$ 15	≤ 2.3	$\begin{cases} 1.997 Z \\ 1.950 E \end{cases}$	—	—	0.097 (3)	—	2.00410	[5, 8]
5a, 5b	$\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}(\text{OH})-\text{CH}_3$ 5c	4 – 11	1.65	1.17	0.177 (3)	0.06 (3)	0.014	2.00302	*
5a, 5b	$\text{CH}_3\text{O}-\text{CH}(\text{OH})-\dot{\text{C}}\text{H}-\text{CH}_3$ 10	2.3 – 9.8	2.21	$\begin{cases} 1.64 \\ 2.60 (3) \end{cases}$	—	0.007 (4) ^g	—	2.00249	*
5a, 5b	$\text{OCH}-\dot{\text{C}}\text{H}-\text{CH}_3$ 13	$\begin{cases} \leq 2.5 \\ 9-11 \end{cases}$	1.79	$\begin{cases} 0.196 \\ 2.143 (3) \end{cases}$	—	—	—	2.00426	* [5, 7]
6a	$\text{CH}_3\text{O}-\text{CH}(\text{OH})-\dot{\text{C}}\text{H}-\text{Cl}$ 11	2 – 9	2.11	1.385	$a_x \begin{matrix} {}^{35}\text{Cl} = 0.30 \\ {}^{37}\text{Cl} = 0.25 \end{matrix}$	—	—	2.00570	*
7a	$\text{HOCH}_2-\dot{\text{C}}(\text{OCH}_3)-\text{CH}_2\text{OH}$ 7c	1.5 – 11	—	0.90 (4) ^b	0.22 (3)	—	0.013 (2)	2.00276	*
7a	$\text{HOCH}_2-\text{CO}-\dot{\text{C}}\text{H}_2$ 16	≤ 2	$\begin{cases} 1.98 Z \\ 1.95 E \end{cases}$	—	—	0.23 (2)	—	2.00414	* [8]
8a, 8b	 8c	4 – 11	—	$\begin{cases} 0.755 (2)^a \\ 2.680 (2) \end{cases}$	0.175 (2)	0.072 (2)	0.020	2.00308	*
8a, 8b	$\text{HOCH}_2\text{CH}_2\text{CH}_2-\text{CO}-\dot{\text{C}}\text{H}_2$ 17	≤ 3	$\begin{cases} 1.99 Z \\ 1.95 E \end{cases}$	—	—	$\begin{cases} 0.150 (2) \\ \delta\text{-H: } 0.036 (2) \\ \epsilon\text{-H: } 0.006 (2) \end{cases}$	—	2.00413	* [8]
9a, 9b	 9c	4 – 12	1.670 ^{c, d}	1.066 ^{c, d}	$\begin{cases} 0.352 \\ 0.108 \end{cases}$	0.115 (2)	0.0 ^f	2.00301	* [11]
	9c ^e		1.631 ^d	1.115 ^d	$\begin{cases} 0.335 \\ 0.107 \end{cases}$	0.115 (2)	0.0 ^f	—	*
9a, 9b	 12	2.3 – 10	2.19	$\begin{cases} 1.720 \\ 3.092 \\ 4.315 \end{cases}$	—	$\begin{cases} 0.062 \\ 0.036 \end{cases}$	0.050	2.0025	* [11]

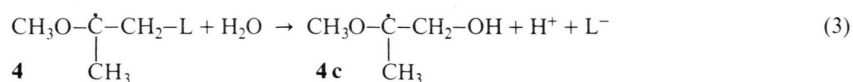
^a The central line of the β -CH₂ triplet is broadened, *cf.* [2].^b The quintet from two β -CH₂-OH groups shows alternating line broadening, see Fig. 4.^c The assignment of a_i to α -H and β -H is based on the signs of the temperature gradients $d|a|/dT$; $a(\alpha\text{-H})$ is assigned to the negative sign and $a(\beta\text{-H})$ to the positive sign on the basis of the known negative sign of $d|a|/dT$ ($\alpha\text{-H}$) of the tetrahydrofuran-2-yl radical [10] and that of two derivatives thereof which are included in Table I, *cf.* notes b and c of Table I.^d $d|a|/dT$ ($\alpha\text{-H}$)/ $dT = -1.24 \mu\text{T/K}$, $d|a|/dT$ ($\beta\text{-H}$)/ $dT = +1.69 \mu\text{T/K}$, *cf.* radical **9b** (Table III).^e At 304 K, see Fig. 2.^f $a(\text{OH}) = 0.052$ and 0.05 mT at 223 K and 251 K respectively, in acetone.^g $a(\delta\text{-OCH}_3)$ (3) and $a(\gamma\text{-OH})$.

* The present work.

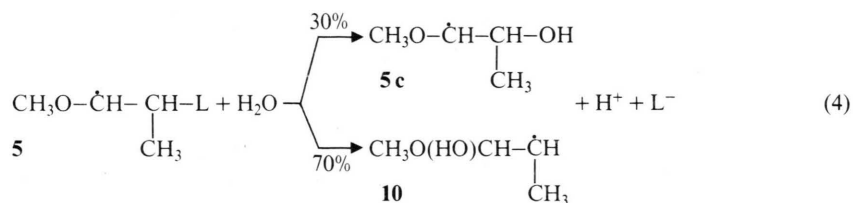


2d, L = Cl; **2f**, L = OPO₃H₂;

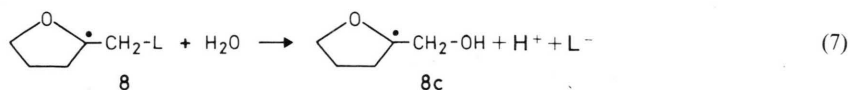
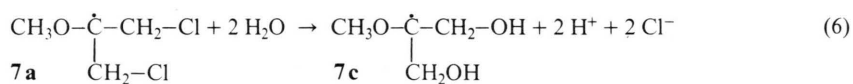
2g, L = OPO₃H(CH₂CH₂OCH₃)



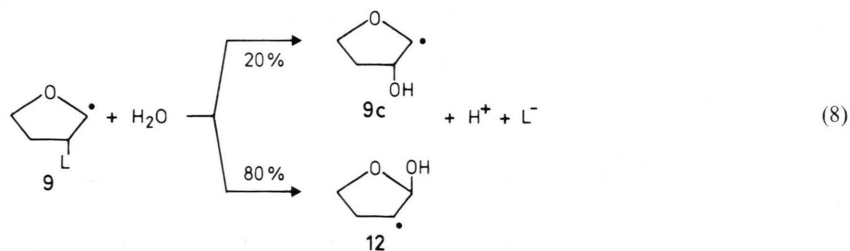
4a, L = Cl; **4b**, L = OCOCH₃



5a, L = Cl; **5b**, L = OCOCH₃

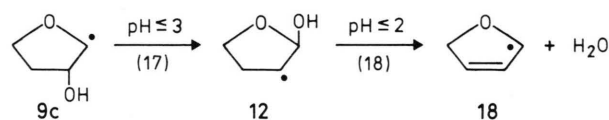
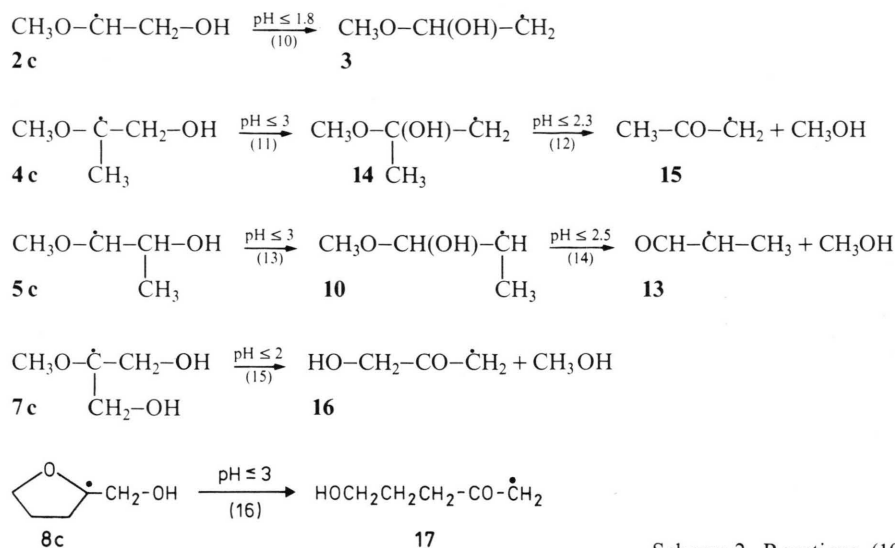


8a, L = Cl; **8b**, L = OCOCH₃



9a, L = Cl; **9b**, L = OCOCH₃

Scheme 1. Reactions (2) to (8). Kinetically controlled product distribution upon hydrolysis of the radicals **2d**, **2f**, **2g**, **4a** to **9a**, **4b**, **5b**, **8b**, and **9b**. The disappearance in the ESR spectra of the radicals **2a** or **2b** according to reaction (1) requires protonation of the monoanion radicals [2], therefore, the species actually decaying are **2f** or **2g** respectively and the observed percentage of **2c** and **3** is related to the decay of the latter radicals, reaction (2).



Scheme 2. Reactions (10) to (18). Radical rearrangement and elimination reactions at $\text{pH} \leq 3$. In the equations the pH is indicated at which the conversions were almost complete. Approximately one pH unit above these values with the radicals **2c**, **4c**, **5c**, **7c**, **8c** and **12** line broadening was observed due to protonation.

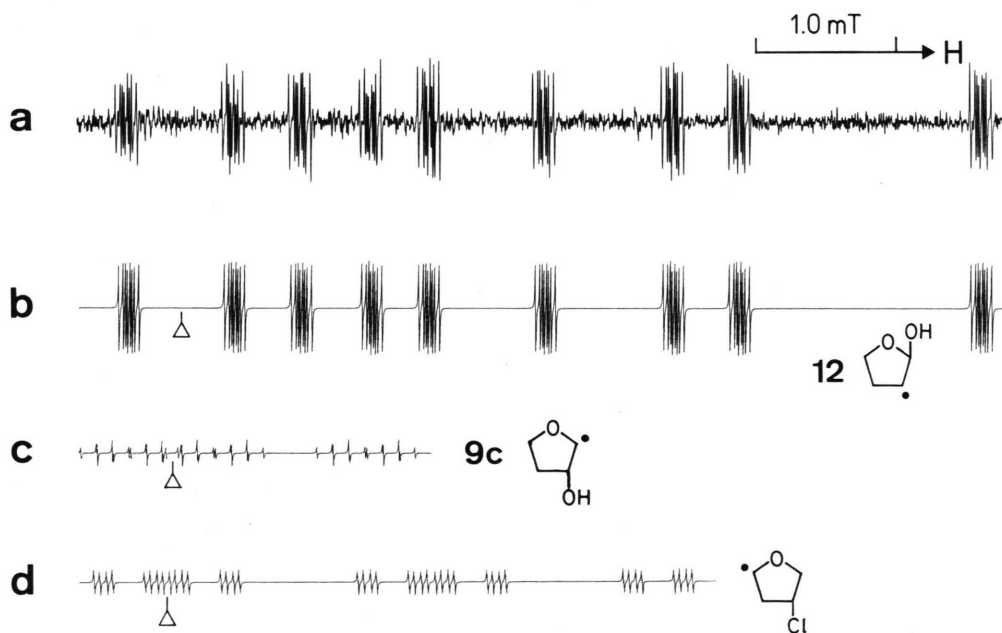


Fig. 1. a) High field part of an ESR spectrum obtained after H-abstraction from 3-chlorotetrahydrofuran, 0.1 M, in aqueous solution, at pH 4, upon u.v. irradiation in a flow system at 276 K, 3 mM $\text{K}_2\text{S}_2\text{O}_8$, and 0.3 M acetone as sensitizer; b), c), and d) are simulated spectra calculated with a line width of 0.005 mT, centers marked by triangles. In a) the lines of **9c** are hard to observe because conversion of **9c** into **12** is already quite effective at pH 4. Under the conditions of high resolution applied here, also the lines of the 5-yl radical (simulated spectrum d) are suppressed because its lines appear broad due to the small Cl splittings that are just about to be separate.

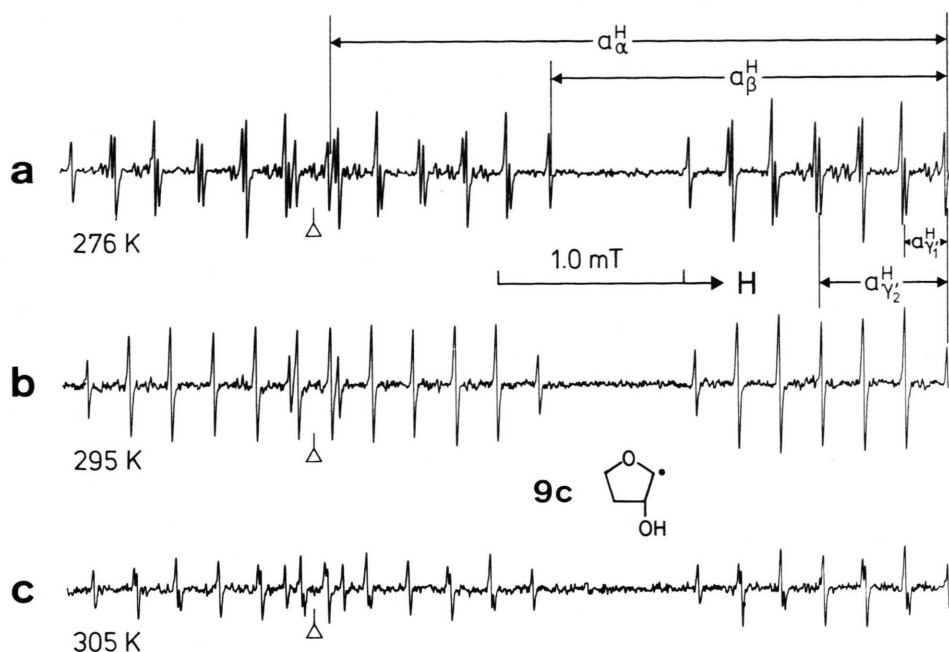


Fig. 2. High field part of ESR spectra of radical **9c** at three different temperatures in water at pH 9.2, 5 mM borate buffer, 0.1 M 3-hydroxytetrahydrofuran as substrate, and otherwise the same conditions as given in Fig. 1. The splittings with α -H and β -H have opposite temperature gradients, *cf.* Table II. The small size of the β -H splitting which increases with rising temperature points to a preferred conformation where the OH substituent is eclipsed with the unpaired electron; consequently, the ring is twisted. The difference of the two γ -H splittings indicates non-planarity of the five membered ring. Centers of the spectra are indicated by triangles.

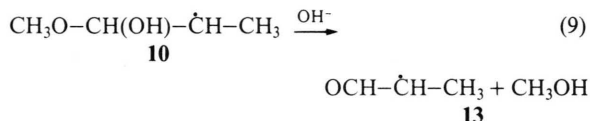
cept for the percentage of the A- and E-type radicals*.

From the results shown in Scheme 1 it is concluded that in each case the product pattern is independent of the nature of the leaving group. The individual pattern is however strongly influenced by the nature of the substituents adjacent to the free spin or to the leaving group: in the reactions (2), (4) and (8) both A- and E-type radicals are formed whereas reactions (3), (6) and (7) gave rise to E-type radicals only and reaction (5) yielded an A-type radical exclusively – as far as can be deduced from ESR spectroscopy.

* Radical ratios were measured by integration of the ESR lines instead of simply comparing the line heights. Thus the influence of the line width variation was eliminated, the line widths of the A-type radicals being often 2 to 6 times larger than those of the isomeric E-type radicals. The rule of thumb holds indeed, that radicals of equal concentration have line heights: $i_{pp} \sim 1/(\delta H)^2$, δH being the half width of the individual line. The half width of sharp lines is however difficult to measure with the required accuracy.

Radicals observed in alkaline solutions at $pH \geq 9$

The signals of radical **10** (Table II) became smaller at $pH \geq 9.5$ and were no longer observable at $pH \geq 10$ *. At $pH \geq 9$ the signals of the radical **13** appeared (Table II) which on increasing the pH increases in intensity to a constant value at $pH \geq 10$. We are therefore dealing with a base catalyzed elimination of CH_3OH according to reaction (9).



The signals of the radical **12** (Scheme 1 and Table II) showed line broadening at $pH \geq 9.5$, the effect becoming more pronounced with increasing pH, the signals of **12** were no longer observed (among the many other lines) at $pH \geq 10$. A successor radical was not detected.

* A similar situation was encountered earlier with radical **3**.

The other radicals included in Scheme 1 and generated by hydrolysis were unaffected in yield and appearance up to pH values of 11 or above (see Table II). Some of the substrates containing acetoxy substituents, *e.g.* 3-acetoxytetrahydrofuran, were found to be alkali sensitive on prolonged standing at pH ≥ 10 and to give rise to ESR lines derived from the corresponding ether alcohols. The corresponding chloro derivatives were sufficiently stable.

Radicals observed in acidic solutions at pH ≤ 3

In more acidic solutions the A- and E-type radicals observed at pH 5–9, Scheme 1, were found to undergo rearrangement and elimination reactions, see reactions (10) to (18), Scheme 2. The ESR parameters of the radicals formed and their pH ranges of observation are included in Table II. The conversion of the radicals **9c** and **12** into **18** in acidic solution was recently reported [11]. The conversion of **2c** into **3** according to reaction (10) was already known [3].

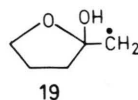
Comments on some structural assignments and some ESR spectra

Radicals **9c** and **12**, reaction (8), have been observed earlier [11] after the reaction of Cl_2^- with 2,3-dihydrofuran in water. There radical **9c** was erroneously considered to be the major species and **12** the minor one. This appears to have been due to the fact that the earlier workers [11] observed only a partial ESR spectrum, because the largest splitting (4.3 mT, Table II) escaped their observation. We resolved and assigned the splittings with all protons of **12**, the full spectrum containing 8 times as many lines (Fig. 1) as could be deduced from the earlier data [11].

With radical **9c** our assignment of the α - and β -H splittings (Table II) is the reverse of the earlier [11] one. Ours is derived from the signs of the temperature gradients observed, Table II and Fig. 2, and it is based on the well established negative sign of the $d|a|(\alpha\text{-H})/dT$ value for the tetrahydrofuran-2-yl radical [10]*. A set of ESR spectra of **9c** at different temperatures was obtained using 3-hydroxytetrahydrofuran as substrate at pH 9, see fig. 2.

* Similar temperature gradients were observed with the cyclic radicals included in Table I and interestingly also with the open chain species **5b**, Table III.

A radical which was observed after H-abstraction from 2-bromomethyl-tetrahydrofuran in aqueous solution at pH ≤ 2 was assigned to radical **19** [9]. The species showed couplings very similar to or identical with ours given in Table II for the radical **17**. The earlier assignment to radical **19** is rejected on the basis of our *g* factor determination. As to the role of radical **19** as intermediate in the conversion of **8c** into **17** see the Discussion.



The “inert” $\dot{\text{C}}\text{H}_2\text{-OR}$ type radicals included in Table I possess non-equivalent α -H's. This is indicated by a moderate broadening of the central lines of the α -triplet at 276 K, see Figs. 3d and 4c. At lower temperatures, see Figs. 5A and 6, the difference in splitting of $\alpha_1\text{-H}$ and $\alpha_2\text{-H}$ is resolved. A similar phenomenon is known for the radical $\dot{\text{C}}\text{H}_2\text{-OCH}_3$ [6].

Radicals observed in acetone as solvent and structure/reactivity relationships

The radicals **4b**, **5b**, **8b**, and **9b** which carry OCOCH_3 as leaving groups and which were not observed in aqueous solution due to their rapid hydrolysis were observable in acetone, Table III and Fig. 6. The corresponding radicals with Cl as leaving group could not be observed; the acetone solutions turned strongly acidic and HCl was found upon wet analysis. One Cl containing radical, probably the least reactive of its kind, viz. the radical **2d**, was observed in acetone, Fig. 5A.

The radicals **2d-g**, **4b** and **8b** which contain the structural element $\text{-O}-\dot{\text{C}}\text{-CH}_2\text{L}$ (L = leaving group) exhibit remarkably low $\beta\text{-CH}_2$ couplings which point to a conformation of the radicals with the leaving group eclipsing the free electron and where the H's of the CH_2 group are bent towards the plane in which the atoms of the group $\text{O}-\dot{\text{C}}-\text{C}$ are located (or H)

cated. The $\beta\text{-CH}_2$ splittings of the radical **2d** are the lowest ones (0.475 mT) ever observed for such a radical. In view of a structure-reactivity correlation presented earlier [2, 12] it is suggested that the radical with the lowest $\beta\text{-CH}_2$ splittings is the

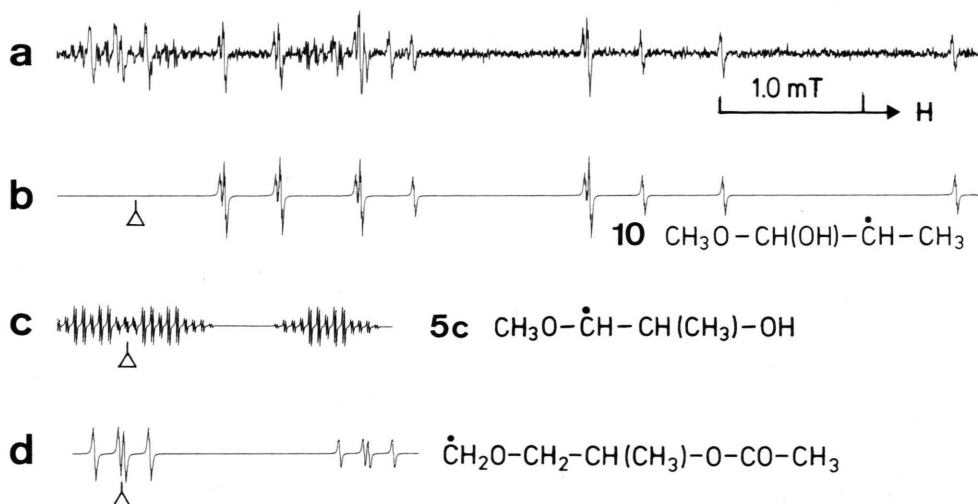


Fig. 3. a) High field part of the ESR spectrum obtained upon UV irradiation of an aqueous solution of 0.1 M 1-methoxy-2-propyl acetate; 3 mM $\text{K}_2\text{S}_2\text{O}_8$, 0.3 M acetone, 5 mM borate buffer, pH 9.0, at 276 K, flow system; b), c) and d) are simulated spectra, centers are indicated by triangles. The prominent lines in Fig. a) belong to the A-type radical **10**, the only one of its kind showing resolved splittings of the remote H's of the OCH_3 and the OH group.

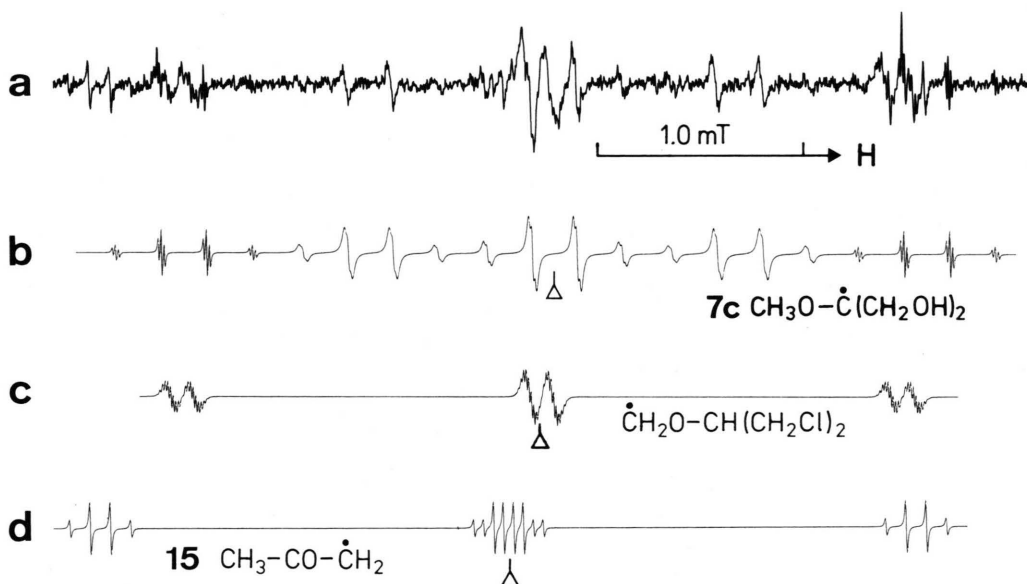


Fig. 4. a) ESR spectrum obtained upon UV irradiation of an aqueous solution of 0.03 M 2-methoxy-1,3-dichloro-propane, containing 4%, v/v, of acetone (to improve the solubility of the substrate), 3 mM $\text{K}_2\text{S}_2\text{O}_8$, borate buffer, pH 7.7, at 276 K, flow system; b), c) and d) are simulated spectra, centers are indicated by triangles. Radical **15** arises from H abstraction at acetone. In Fig. a) and b) the outer quartets of **7c** (from OCH_3) show two well resolved OH splittings (the small triplet). In the next inner quartet these triplet components are poorly resolved and they are moderately resolved at the center. The reason for these line width alternations is hindered rotation of the CH_2OH groups both of which have a preferred conformation where the OH substituents eclipse the unpaired electron. Each of the groups should give rise to a triplet with a broadened center (*cf.* radical **2d**, Fig. 5A) and the combination of two such identical groups gives the quintet pattern with the line width alternation observed. Fig. c) is a composite of spectra of the isotopic radicals $\dot{\text{R}}(^{35}\text{Cl})_2$, $\dot{\text{R}}(^{35}\text{Cl}^{37}\text{Cl})$ and $\dot{\text{R}}(^{37}\text{Cl})_2$ in the ratio 0.56 : 0.375 : 0.063.

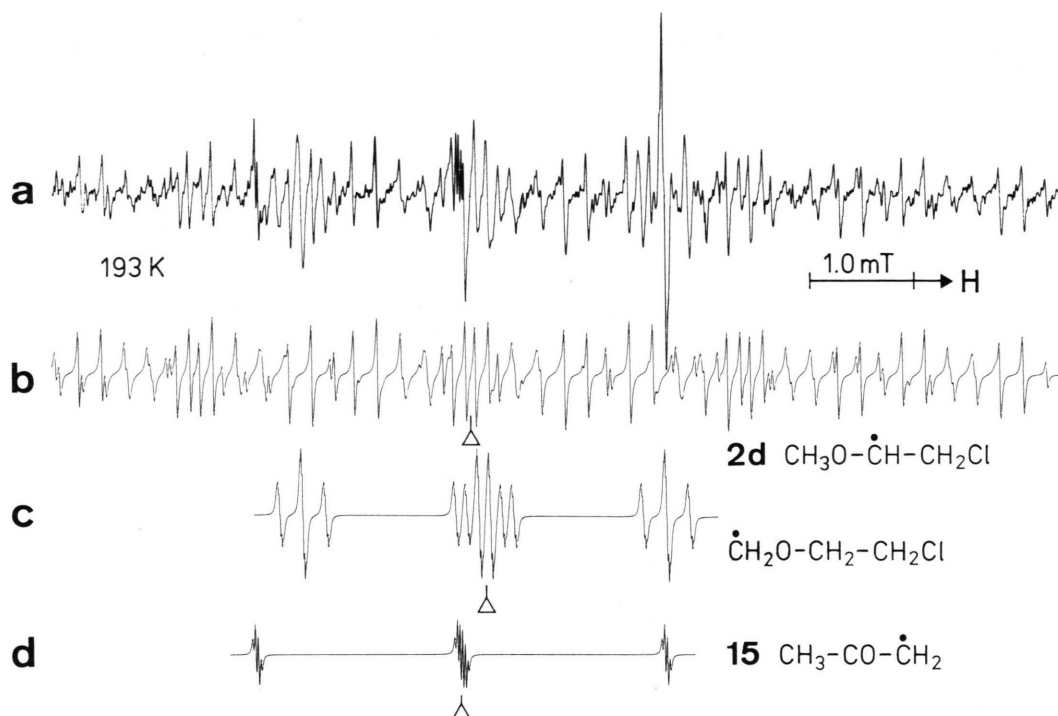


Fig. 5A. a) ESR spectrum obtained upon UV irradiation in a flow system of an acetone solution of 0.2 M 2-methoxyethyl chloride and 0.1 M *tert.*-butyl peroxide, at 193 K; b), c) and d) are simulated spectra, the center is indicated by a triangle. In a) the central line of the small triplet (*cf.* Table III) shows selective broadening, which under conditions of high resolution would be buried in the noise; the spectrum a) is therefore overmodulated and stressed to 0.02 mT line width in order to present all lines. Fig. b) is a composite of spectra of the isotopic radicals $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2^{35}\text{Cl}$ and $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2^{37}\text{Cl}$, 3 : 1, assuming the $a(\beta\text{-H})$ triplet to have line heights of 1 : 0.5 : 1 and line widths of 1 : 2 : 1. Details of the calculation (or the analysis of the spectrum) are given in Fig. 5B. With the radical $\dot{\text{C}}\text{H}_2\text{O}-\text{CH}_2-\text{CH}_2\text{Cl}$, *cf.* c), the non-equivalent $\alpha\text{-H}$ splittings, 1.81 and 1.70 mT, are resolved; they are averaged at 276 K, *cf.* Table I. A similar behaviour was reported for the methoxymethyl radical [6]. The acetonyl radical, **15**, arises from H abstraction at acetone; the right hand lines overlap accidentally with lines of other components to give the huge line in the spectrum a).

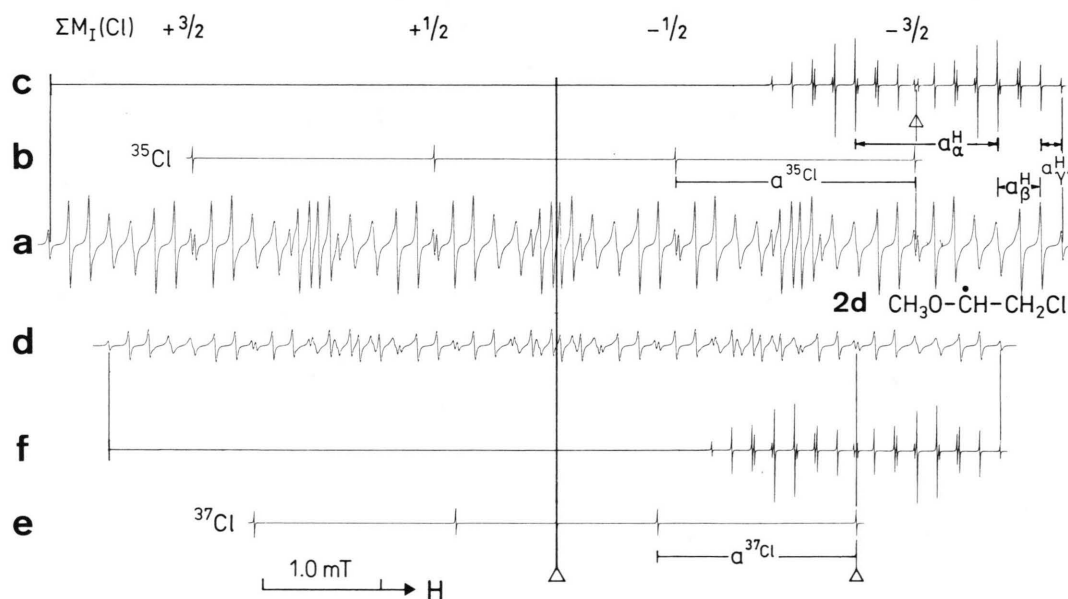


Fig. 5B. Spectrum b) in Fig. 5A is the composite of the spectra a) and d) of Fig. 5B. Fig. a) is constructed from the ^{35}Cl quartet of **2d**, Fig. b), and four times the partial spectrum of the structure $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2-$, one of which is shown as example (but without line broadening) at the right hand of Fig. c). The $a(\gamma\text{-H})$ quartet, the $a(\beta\text{-H})$ triplet and the $a(\alpha\text{-H})$ doublet are indicated in Fig. c). Fig. d) is composed likewise from the (smaller) ^{37}Cl quartet, Fig. e) and the same partial spectrum as above, Fig. f), where line broadening is again omitted.

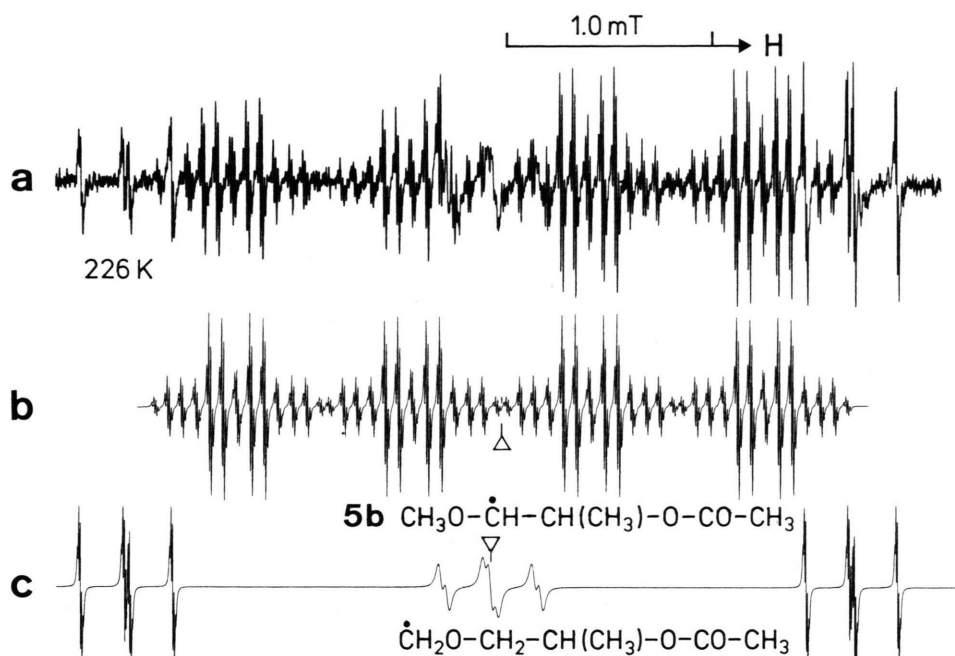


Fig. 6. a) ESR spectrum obtained upon UV irradiation of an acetone solution of 0.1 M 1-methoxy-2-propyl acetate and 0.1 M *tert.*-butyl peroxide at 226 K; b) and c) are simulated spectra. In the radical **5b** the OCOCH_3 group eclipses the unpaired electron as is concluded from the low a (β -H) value, its temperature dependence (*cf.* Table III) and the fact that all splittings with H's are resolved. The radical of spectrum c) has two non-equivalent α -H's, just about to be resolved at the temperature chosen. At higher temperatures (*cf.* Fig. 3) the spectrum become triplet shaped, because the different α -splittings average due to increased rates of rotation within the radical. Interestingly, the non-equivalence of the two γ -H's remains almost independent of the temperature, *cf.* Table I, as may be seen from the outermost doublets.

most reactive one with respect to hydrolysis. Another characteristic of these radicals is selective line broadening, see Fig. 5A and Tab. III. Interestingly, selective line broadening and small β -splittings were also observed with the corresponding radicals with $\text{L}=\text{OH}$, *cf.* Fig. 4 and Table II; therefore the OH group also eclipses the unpaired electron. Analogous conformations are assumed for the radicals **5b**, **5c**, **9b** and **9c** because of their small β -H splittings and similar temperature gradients, *cf.* Tables II and III.

Chemical analysis

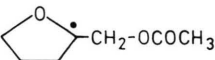
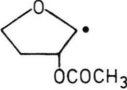
The substrates used for ESR spectroscopy were also subjected to ^{60}Co - γ -irradiation in N_2O -saturated aqueous solutions at concentrations of 5×10^{-3} to 10^{-2} M at 274 K. Subsequently H^+ and Cl^- were determined quantitatively by potentiometric titration; CH_3OH was determined by g.l.c., Table IV. The product concentrations were found to increase linearly with dose. The doses applied caused a

maximum conversion of substrate of 10%. The yields per 100 eV of energy absorbed (the G -values) were found to be independent of dose as well as of dose rate. The latter finding excludes contributions to the yields from chain reactions. The yields are given in G values and in terms of f , the yield in percentage of substrate radicals formed by reaction with OH and H, using a G value for the formation of substrate radicals of 6.6 (Table IV). The f -value for H^+ is then taken as the percentage of hydrolysing radicals among the total of H-abstraction radicals formed. Therefore, the f -value is a measure of the H abstraction by $\cdot\text{OH}$ at the β -position of the substrate versus all other positions.

Scavenging experiments with oxygen

Some scavenging experiments with oxygen were also carried out. For this purpose the solutions were purged with a stream of $\text{N}_2\text{O}/\text{O}_2$, 4/1 (v/v) during γ -irradiation. Under these conditions the hydrated electrons primarily formed upon radiolysis of water

Table III. ESR parameters of some α -alkoxy alkyl radicals carrying a leaving group in β -position, observed in acetone. Hyperfine splittings in mT, number of protons larger than 1 in parentheses.

Radical	a_{α}^H	a_{β}^H	a_{γ}^H	Other splittings	g Factor	Temp. [K]
$\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2-\text{Cl}$ 2d	$\left\{ \begin{array}{l} 1.56 \\ 1.58 \end{array} \right.$	$\left\{ \begin{array}{l} 0.50 \text{ (2)}^a \\ 0.475 \text{ (2)}^a \end{array} \right.$	$\left\{ \begin{array}{l} 0.21 \text{ (3)} \\ 0.224 \text{ (3)} \end{array} \right.$	$\begin{array}{l} {}^{35}\text{Cl} \quad 2.58^b \\ {}^{37}\text{Cl} \quad 2.15^b \\ {}^{35}\text{Cl} \quad 2.660 \\ {}^{37}\text{Cl} \quad 2.214 \end{array}$	$\begin{array}{l} \\ 2.00353 \end{array}$	$\begin{array}{l} 274 \\ 193 \end{array}$
$\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2-\text{OPO}_3\text{H}_2$ 2f	1.750	0.636 (2) ^a	0.217 (3)	P	0.475	2.00305 237
$\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2-\text{OPO}_3\text{H}(\text{CH}_2\text{CH}_2\text{OCH}_3)$ 2g	1.75	0.623 (2) ^a	0.217 (3)	P	0.505	2.00304 243
$\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2-\text{OCOCH}_3$ 2e	1.73	0.765 (2) ^a	0.203 (3)	OAc	0.01 (3)	2.00303 276
$\text{CH}_3\text{O}-\dot{\text{C}}(\text{CH}_3)-\text{CH}_2-\text{OCOCH}_3$ 4b	$\left\{ \begin{array}{l} - \\ - \end{array} \right.$	$\left\{ \begin{array}{l} 0.67 \text{ (2)}^a \\ 2.05 \text{ (3)} \end{array} \right.$	$\left\{ \begin{array}{l} 0.215 \text{ (3)} \end{array} \right.$	OAc	0.01 (3)	2.00303 276
$\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}(\text{CH}_3)-\text{OCOCH}_3$ 5b	$\left\{ \begin{array}{l} 1.664^c \\ 1.695 \end{array} \right.$	$\left\{ \begin{array}{l} 0.895^c \\ 0.845 \end{array} \right.$	$\left\{ \begin{array}{l} 0.195 \text{ (3)} \\ 0.202 \text{ (3)} \end{array} \right.$	$\begin{array}{l} \text{OAc} \quad 0.01 \text{ (3)} \\ \gamma\text{-H} \quad 0.064 \text{ (3)} \\ \text{OAc} \quad 0.010 \text{ (3)} \\ \gamma\text{-H} \quad 0.064 \text{ (3)} \end{array}$	$\begin{array}{l} 2.00303 \\ \\ \end{array}$	$\begin{array}{l} 276 \\ 226 \end{array}$
8b 	$\left\{ \begin{array}{l} - \\ - \end{array} \right.$	$\left\{ \begin{array}{l} 0.653 \text{ (2)}^a \\ 2.69 \text{ (2)} \end{array} \right.$	$\left\{ \begin{array}{l} 0.202 \text{ (2)} \end{array} \right.$	$\begin{array}{l} \text{OAc} \quad 0.012 \text{ (3)} \\ \gamma\text{-H} \quad 0.072 \text{ (2)} \end{array}$	$\begin{array}{l} 2.00309 \end{array}$	$\begin{array}{l} 264 \end{array}$
9b 	$\left\{ \begin{array}{l} 1.71^d \\ 1.75 \end{array} \right.$	$\left\{ \begin{array}{l} 0.99^d \\ 0.95 \end{array} \right.$	$\left\{ \begin{array}{l} 0.410 \\ 0.105 \\ 0.430 \\ 0.090 \end{array} \right.$	$\begin{array}{l} \text{OAc} \quad 0.01 \text{ (3)} \\ \gamma\text{-H} \quad 0.13 \text{ (2)} \\ \text{OAc} \quad 0.01 \text{ (3)} \\ \gamma\text{-H} \quad 0.14, 0.13 \end{array}$	$\begin{array}{l} 2.00303 \end{array}$	$\begin{array}{l} 261 \\ 223 \end{array}$




^a *cf.* Table II, note a, at lower temperature the line broadening is increased (see Fig. 5A), *cf.* also [2].^b The outer lines generated from $M_1 + 3/2$ and $-3/2$ Cl multiplets are extensively broadened above 240 K.^c $d|a|(\alpha\text{-H})/dT = -0.62 \mu\text{T/K}$ and $d|a|(\beta\text{-H})/dT = +1.00 \mu\text{T/K}$.^d $d|a|(\alpha\text{-H})/dT = -1.05 \mu\text{T/K}$, $d|a|(\beta\text{-H})/dT = +1.05 \mu\text{T/K}$, *cf.* radicals **5b**, **9c**, and Table II, note c.^e Data added for comparison.

are still scavenged by the N_2O to form OH radicals, *cf.* Experimental, but the radicals formed after reaction of the OH radicals with the substrates will eventually be scavenged by the O_2 , unless they are too short-lived to be scavenged. The results are included in Table IV. Comparing the $G(\text{Cl}^-)$ -values under $\text{N}_2\text{O}/\text{O}_2$ with those under N_2O it is seen that (except for $\text{CH}_3\text{O}-\text{CH}_2-\text{CHCl}_2$) the yields of Cl^- are the same within the limits of error or slightly larger under $\text{N}_2\text{O}/\text{O}_2$. A slight increase of the yield of Cl^- under $\text{N}_2\text{O}/\text{O}_2$ is not due to a contribution of chain reactions since dose rate effects were absent; it is assigned to the contribution to the yield of Cl^- from α -chloroalkyl radicals under these conditions. Such radicals are formed as minor components in the reactions of OH radicals with the substrates. After addition of O_2 , followed by bimolecular radical-radical termination and consecutive reactions, Cl^- is eventually eliminated. That Cl^- is quantitatively eliminated is well illustrated by the behaviour of $\text{CH}_3\text{OCH}_2\text{CHCl}_2$ as substrate. $G(\text{Cl}^-)$ under $\text{N}_2\text{O}/\text{O}_2$ is twice as large as found under N_2O

(Table IV). The elimination of the first Cl^- under N_2O is due to the hydrolysis of radical **6a**, reaction (5). From the doubling of the yield of Cl^- in the presence of N_2O and O_2 three conclusions are drawn: i) the elimination of the first Cl^- is so fast that it is not inhibited by O_2 , ii) hydrolysis of the radical **6a** yields the A-type radical **11** exclusively (in agreement with the results obtained from ESR spectroscopy); the formation of the E-type radical $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CHCl}(\text{OH})$ would have led inevitably to the loss of the second Cl as HCl and if this process were significant the yield of Cl^- under N_2O should have been larger than half of the yield under $\text{N}_2\text{O}/\text{O}_2$ which was not the case, iii) attachment of O_2 to the α -chloroalkyl radical **11** in aqueous solution is followed by quantitative elimination of the α -chlorine as Cl^- . A similar situation was encountered in the reaction of O_2 with the radical cation $(\text{CH}_3\text{O})_2\dot{\text{C}}-\dot{\text{C}}\text{HCl}$ [13].

The failure to inhibit the elimination of (the first) Cl^- on scavenging by O_2 is taken as basis for an

Table IV. Chemical product analysis after ^{60}Co - γ -irradiation of aqueous solutions of selected β -substituted alkyl ethers as substrates, 5×10^{-2} M, at 273 K and saturated with the gases specified. The doses applied caused maximal conversion of substrate of 10%. G -values = yields of molecules per 100 eV of energy absorbed in the solution; f -value = percentage of hydrolyzing radicals among the total of radicals formed by reaction of $\cdot\text{OH}$ or H^\cdot with the substrate, calculated from $\bar{G}(\text{H}^+)$ on the basis of 6.6 substrate radicals formed per 100 eV. OAc = OCOCH_3 .

Substrate	Gas	pH	$G(\text{H}^+)$	$f(\text{H}^+)$	$G(\text{Cl}^-)$	$G(\text{CH}_3\text{OH})$	$G(\text{others})$
$\text{CH}_3\text{O}-\text{CH}_2-\text{CH}_2-\text{Cl}$	N_2O	3.5–7	3.5 ± 0.1	53 ± 2	3.5 ± 0.1	1.4 ± 0.2	CH_3CHO 0.5 ± 0.1 $(\text{CH}_2\text{CHO})_2$ 0.007 ± 0.002
	N_2O	7 *	—	—	3.4 ± 0.1	1.4 ± 0.2	CH_3CHO 0.5 ± 0.1
	N_2	2	—	—	3.8 ± 0.1	3.6 ± 0.3	CH_3CHO 0.7 ± 0.1 $(\text{CH}_2\text{CHO})_2$ 0.7 ± 0.04
	$\text{N}_2\text{O}/\text{O}_2$ 4:1	3	—	—	3.9 ± 0.1	—	$\text{CH}_2\text{OH}-\text{CHO}$ 1.1
$\text{CH}_3\text{O}-\text{CH}_2-\text{CH}_2-\text{OAc}$	N_2O	4–7	2.9 ± 0.1	44 ± 2	—	1.2 ± 0.2	CH_3CHO 0.4 ± 0.1 $(\text{CH}_2\text{CHO})_2$ 0.05 ± 0.01
$\text{CH}_3\text{O}-\underset{\text{CH}_3}{\text{CH}}-\text{CH}_2-\text{OAc}$	N_2O	4–7	2.5 ± 0.1	38 ± 2	—	1.7 ± 0.2	CH_3COCH_3 0.25 ± 0.05
$\text{CH}_3\text{O}-\underset{\text{CH}_3}{\text{CH}}-\text{CH}-\text{OAc}$	N_2O	4–7	3.1 ± 0.1	47 ± 2	—	2.8 ± 0.2	$\text{CH}_3\text{CH}_2\text{CHO}$ 0.8
$\text{CH}_3\text{O}-\underset{\text{Cl}}{\text{CH}}-\text{CH}_2-\text{Cl}$	N_2O	3	3.8 ± 0.1	58 ± 2	3.7 ± 0.1	3.2 ± 0.2	—
	$\text{N}_2\text{O}/\text{O}_2$ 4:1	3	8.0 ± 0.2	—	7.2 ± 0.2	—	—
$\text{CH}_3\text{O}-\underset{\text{CH}_2-\text{Cl}}{\text{CH}}-\text{CH}_2-\text{Cl}$	N_2O	3.5	4.6 ± 0.1	35 ± 1	2.2 ± 0.1	—	—
	N_2O	3.5–7	2.3 ± 0.1	35 ± 1	2.3 ± 0.1	—	—
	$\text{N}_2\text{O}/\text{O}_2$ 4:1	3.5–7	3.2 ± 0.1	—	2.4 ± 0.1	—	HCHO 0.7 ± 0.1
	N_2O	4–7	1.8 ± 0.1	27 ± 1	—	—	—
	N_2O	4–7	2.2 ± 0.1	34 ± 1	—	—	—

* Phosphate buffered solution.

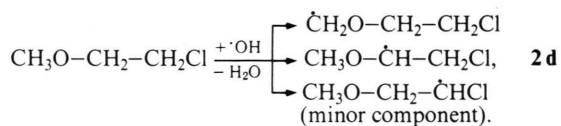
estimate of a minimal rate constant of decay of the β -chlorine substituted E-type radicals in aqueous solution. Considering the rate of hydrolysis to be at least 10 times faster than the rate of reaction with O_2 it is:

$$k(\text{hydrol.}) \geq 10 \times [\text{O}_2] \times k(\dot{\text{R}} + \text{O}_2).$$

Taking a value for the rate constant of the reaction of the radicals with O_2 close to the diffusion controlled limit, $k(\dot{\text{R}} + \text{O}_2) = 5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and $[\text{O}_2] = 4.3 \times 10^{-4} \text{ M}$, at 0.2 atm O_2 and 273 K it follows that $k(\text{hydrol.}) \geq 2.1 \times 10^7 \text{ s}^{-1}$, corresponding to a half life in H_2O of $\tau_{1/2} \leq 35$ nanoseconds.

Discussion

Hydrogen abstraction from β -acetoxy or β -chloro substituted alkyl ethers by $\cdot\text{OH}$, $\text{SO}_4^{\cdot-}$ or $(\text{CH}_3)_3\text{C}-\text{O}^\cdot$ radicals is expected to lead to all possible H-abstraction radicals, for example:



The main components are **2d** and $\dot{\text{C}}\text{H}_2\text{O}-\text{CH}_2-\text{CH}_2\text{Cl}$, the minor component is $\text{CH}_3\text{O}-\text{CH}_2-\dot{\text{C}}\text{HCl}$. Therefore, hydrogen abstraction is particularly effective at either carbon in α -position to the ether oxygen as

is *e.g.* seen from the ESR spectra in acetone solution, Figs. 5A and 6 and Table III. In aqueous solution, however, the β -chloro or acetoxy substituted alkyl ether radicals are not observed, their hydrolysis products appear instead, Table II, Figs. 1–4. The fast changes in electrical conductivity observed after pulse radiolysis are therefore assigned to the formation of acid by the hydrolysis reactions of the β -substituted radicals, Scheme 1. From the rate of the conductivity increase (see Results, chapter pulse radiolysis) it follows that all the β -substituted radicals, including **2d**, hydrolyze with a half life of less than one microsecond, whereas the radical $\text{CH}_3\text{O}-\dot{\text{C}}\text{H}-\text{CH}_2\text{OCOCH}_3$, **2e**, hydrolyses with a half life of about 350 microseconds*. In view of the model character for DNA-4' radicals the product patterns of hydrolysis, Scheme 1, the subsequent acid catalyzed OH shifts, the elimination of CH_3OH or H_2O or a corresponding ring opening reaction, Scheme 2, are of importance. In the following each of these aspects will be discussed separately and appropriate reference will be made as to the reaction mechanisms. The behaviour of the model radicals is then used to advance a (modified) scheme of degradation of DNA-4' radicals.

A) The hydrolysis reactions

Kinetic or thermodynamic product control

The product patterns of hydrolysis observed by ESR spectroscopy at pH 5–9 are shown in Scheme 1. In the reactions (2), (4) and (8) of that scheme both A- and E-type radicals are formed. The A-type radicals are known to be the thermodynamically more stable ones of the two [3]. In accord with this the E-type radicals are generally converted into the A-type ones under proton catalysis, see Scheme 2. It is then assumed that the A-type to E-type ratios observed at pH 5–9 are the result of kinetically controlled substitution reactions by H_2O and that thermodynamic product control sets in at $\text{pH} \leq 3$.

* Since under ESR conditions the first half life of such radicals with respect to bimolecular radical-radical termination is about 100–300 microseconds it is now apparent why **2e** but not **2d** is observed [5] in aqueous solution.

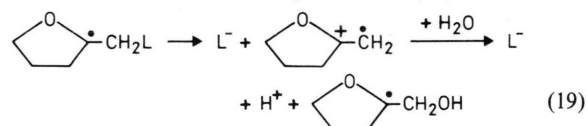
Factors governing the regio selectivity

Under kinetic product control the steric effects of substituents at the carbon atom carrying the leaving group are of major importance in directing the incoming OH group (compare reactions (2), (4), (5), and (8)). Electronic factors are also involved as may be seen from a comparison of the effects of substitution by CH_3 or Cl – two equally sized substituents – which lead to 30 and zero percent entry of OH at the site of the leaving group, reactions (4) and (5).

Upon substitution of H at the radical site of radicals **2**, proceeding to the radicals **4**, **7**, or **8**, the probably steric influence of the substituents outweighs any other effect and the entry of OH at the position of the leaving group is now 100% or regio specific (within the limits of ESR observation).

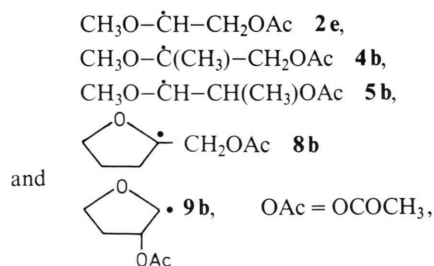
The mechanism of the hydrolysis reaction

As detailed below evidence is accumulated that the hydrolysis of the model radicals is a two-step process with alkoxyalkene radical cations as short-lived intermediates, see *e.g.* reaction (19).



The regio selectivities presented in Scheme 1 are therefore the regio selectivities of the addition of water to the intermediate radical cation followed or accompanied by the loss of a proton. Part of the evidence for the existence of an intermediate is derived from the Results presented in this paper and will be pointed out below. Other parts of the evidence will be submitted for publication elsewhere [14] but they will be referred to briefly.

Comparing the rates of hydrolysis of the radicals



it is seen that substitution of H in **2e** by CH_3 or an alkyl group at either the radical site or at the carbon atom carrying the OAc group is accompanied by an

increase in hydrolysis rate by a factor ≥ 350 . This large methyl or alkyl group effect is in accord with a rather polar transition state of hydrolysis of these radicals. The effect is in accord with an S_N1 mechanism of hydrolysis which involves the formation of a radical cation as intermediate. An S_N2 mechanism of nucleophilic substitution by water is excluded by this effect since in that case rate retardation is expected (or only small changes rather than the observed large rate acceleration) because of the steric effects of CH_3 or alkyl groups. Also in accord with a strongly polar transition state is the large acceleration of hydrolysis rate observed earlier [2] upon protonation or alkylation of phosphate as leaving group in radical **2**. Further in accord with this is the difference observed in this work in hydrolysis rates of the radicals **2d** and **2e**. A more quantitative treatment of the effects of substitution at the organic skeleton of the radicals or at their leaving groups on the rates of hydrolysis will be given elsewhere [14].

In agreement with the assumption of radical cations as intermediates is the observation that the (kinetically controlled) A-type/E-type ratios, Scheme 1, are independent of the nature of the leaving group. As will be shown in a subsequent paper these ratios are also independent of the nature of the incoming nucleophile [14]. Kinetic evidence for the existence of intermediates is derived from the facts that with increasing reactivity towards nucleophilic attack, as observed in the present work, selectivity with respect to the nucleophiles is also increased [14]. The half lives of the alkoxyalkene radical cations in water are estimated to be in the order of nanoseconds.

Substitution of an H-atom at the radical site of **2d** by a second methoxyl group leads to the radical $(\text{CH}_3\text{O})_2\dot{\text{C}}\text{--CH}_2\text{Cl}$ which in aqueous solution dissociates into Cl^- and a radical cation, see reaction (20).



This radical cation is so stable in aqueous solution that its ESR spectrum could be measured [15]. This is considered as further indication for the intermediacy of radical cations in the cases presented in Scheme 1.

Influence of the solvent on the rate of formation of radical cations

Another important contribution to the high rates of hydrolysis is the solvation by water. This is well

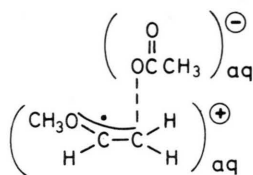


Fig. 7. Structure assumed for the transition state of hydrolysis of the radical **2e**. The structure of the radical moiety closely resembles the structure of the methoxyethylene radical cation. The incipient ions are partially solvated. The acetate group is assumed to lie above the plane

defined by the atoms $\begin{array}{c} \text{O} \\ \diagup \\ \text{C--C} \\ \diagdown \\ \text{H} \end{array}$ but its exact position above this plane is not known.

born out by the fact that some of the radicals suffering fast hydrolysis in aqueous solution are sufficiently stable in acetone, *cf.* Table III. Obviously the formation of ions – the radical cation and L^- , *cf.* *e.g.* reaction (19) – is much easier in water than in acetone.

The solvation of ions is accompanied by negative reaction entropies and significant contributions of the solvent to the structure of the transition states, as *e.g.* in S_N2 -type hydrolysis reactions, usually go along with negative activation entropies. On the other hand activation entropies for S_N1 -type hydrolysis reactions are (typically) positive, probably mainly due to the release of steric strain in such processes. A typical example is the activation entropy for the hydrolysis of *tert.*-butyl chloride in water, $\Delta S^\ddagger = +35 \text{ J/K mol}$ [16]. The activation parameters of the hydrolysis of radical **2e** in aqueous solution were determined to be $\Delta H^\ddagger = 52 \pm 6 \text{ kJ/mol}$ and $\Delta S^\ddagger = +4 \pm 20 \text{ J/K mol}$ [17]. Since hydrolysis of the radical **2e** is S_N1 in nature (the formation of the radical cation being rate limiting) the less positive ΔS^\ddagger -value associated with this reaction as compared with those observed with other S_N1 reactions is now understood as non-nucleophilic solvent assistance. The structure of the transition states of hydrolysis of our model radicals is then tentatively described as shown in Fig. 7.

B) Proton catalyzed OH shifts

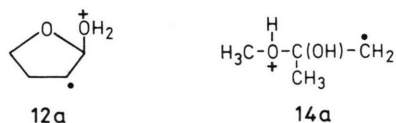
E-type radicals rearrange to A-type ones on proton catalysis. In the present work three such cases have been established, reactions (11), (13), and (17). An early example of a reaction of this type is the H^+ catalyzed rearrangement of $\text{CH}_3\text{O--}\dot{\text{C}}\text{H--CH}_2\text{OH}$

into $\text{CH}_3\text{O}-\text{CH}(\text{OH})-\dot{\text{C}}\text{H}_2$ [3], already mentioned in the Introduction. Recent examples are the proton catalyzed rearrangement reactions of some β -hydroxyalkyl radicals, *e.g.* of $\dot{\text{C}}\text{H}_2\text{C}(\text{OH})(\text{CH}_3)_2$ into $\text{HO}-\text{CH}_2-\dot{\text{C}}(\text{CH}_3)_2$ [18]. For both cases radical cations have been invoked [18] as intermediates.

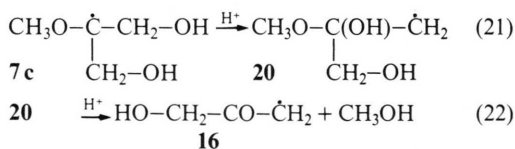
A mechanistic alternative has been proposed by Golding and Radom [19] in that the reaction passes through a polar transition state with the organic skeleton resembling in structure the radical cation, but by-passing the radical cation as true intermediate. The authors [19] calculated rather low activation energies for 1,2-shifts of protonated OH groups and predicted such processes to be readily observable.

C) Proton catalyzed elimination and ring opening reactions

The A-type radicals undergo further changes in more acidic solutions, *cf.* Scheme 2. A proton catalyzed elimination of methanol is observed from radical **10**, reaction (14), and from radical **14**, reaction (12). From radical **12** a proton catalyzed elimination of water to yield the allyl-type radical **18*** is observed, reaction (18). Radical cations like *e.g.* **12a** or **14a** are assumed as intermediates.

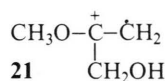


It is assumed that also the elimination of CH_3OH from the E-type radical **7c**, reaction (15), consists mechanistically of two steps, reactions (21) and (22) with the A-type radical **20** as intermediate.

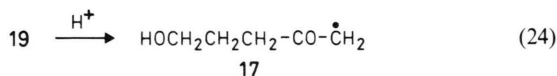
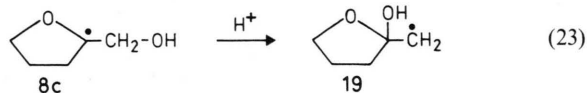


* The formation of radical **18** in acidic solution from the radicals **9c** and **12** was suggested earlier [11], however the formation of the allyl type radical in the presence of 2,3-dihydrofuran left some doubt as to the genesis of that radical. The present observation and the formation of **18** from non olefinic precursors is in accord with the earlier suggestion.

The basis for this assumption is the analogous behaviour of the radicals **4c** and **14**, reactions (11) and (12), together with the narrow pH range of observation of 2.0 to 3.3 of radical **14**, *cf.* Table II. The failure to observe the A-type radical **20** by ESR spectroscopy is not attributed to thermodynamic instability of the A-type radical **20** with respect to its E-type precursor **7c** but to the kinetic instability of **20** with respect to the proton catalyzed methanol elimination, reaction (22), which may be taken as irreversible under the reaction conditions. The proton catalyzed elimination of H_2O from radical **20** may also occur, but this reaction may be considered as "reversible", since **20** will be formed again through the radical cation **21** and the radical **7c** in successive steps.

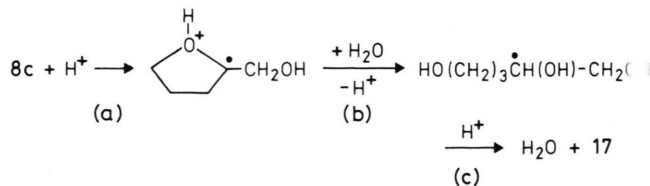


A similar situation is assumed to prevail with the E-type radical **8c** which from ESR observation undergoes ring opening, reaction (16), Scheme 2. A proton catalyzed OH shift, reaction (23), is assumed to precede the ring opening, reaction (24), which is analogous to the methanol elimination reactions (10), (12), (14), and (22).



Radical **19** is not considered thermodynamically unstable with respect to radical **8c** but kinetically unstable with respect to reaction (24).

An alternative explanation for the observed ring opening reaction (16) is disfavoured. This consists of three successive steps, (a), (b), and (c):



Step (a) is certainly fast enough to permit the appearance of **17** under our conditions. That step (c) is also fast enough was checked with the H-abstraction radicals of butanediol-1,2 which suffered proton

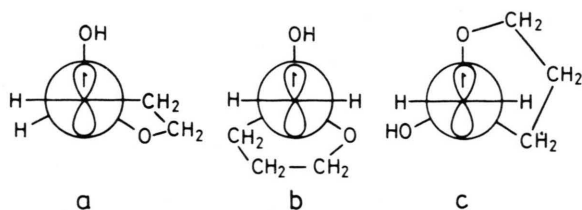
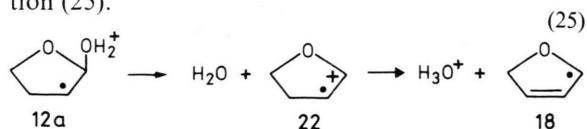


Fig. 8. Newman projections. a: radical **12**; b: radical **19** with the OH group eclipsed with the orbital of the electron; c: **19** with the ether oxygen eclipsed with the radical electron.

catalyzed loss of water already at pH values above 4. Step (b) however is considered to be too slow in this sequence. An analogous hydrolytic cleavage of the tetrahydrofuran ring could not be observed with the 2-tetrahydrofuranyl radical even at pH 1, and there is no reason to be seen why step (b) should then be a fast reaction. Should hydrolytic cleavage of the tetrahydrofuran ring nevertheless be a fast reaction one would also expect the radical **9c** to undergo such a reaction which, however, is not observed.

The acceptance of reactions (23) and (24) as explanation for the observed behaviour of radical **8c** in acidic solution requires however an explanation why the A-type radical **19** undergoes ring opening and why the A-type radical **12** does not, but loses H_2O instead, reaction (18). The reasons appear to be stereo electronic in nature. The best conformation for both, ring opening or H_2O elimination is one in which the orbital of the electron is eclipsed with the leaving group. In radical **12** the OH group adopts this conformation, *cf.* Fig. 8a, as may be inferred from the relatively large OH-splitting of 0.05 mT observed by ESR spectroscopy, *cf.* Table II. The same conformation is assumed for the OH group after protonation, radical **12a**. Since an eclipsed conformation cannot be adopted by the ring oxygen only the elimination of H_2O is favoured* and the resulting radical cation **22** may undergo readdition and elimination of water and eventually

deprotonate to yield the allyltype radical **18**, reaction (25).



In radical **19** the $\dot{\text{C}}\text{H}_2$ group is probably freely rotating, and thus either oxygen may come into an eclipsed position to the radical electron, Figs. 8b and 8c, and on protonation of the OH group H_2O is eliminated and on protonation of the ring oxygen ring opening ensues. The radical cation $\dot{\text{C}}\text{H}_2-\dot{\text{C}}(\text{OH})-\text{CH}_2\text{CH}_2\text{CH}_2-\text{OH}$ which is a plausible intermediate will undergo fast deprotonation to yield the observed radical **17**. The reverse sequence, reprotonation of **17** at the carbonyl group followed by ring closure is too inefficient in slightly acidic aqueous solutions and therefore the ring opening reaction is practically irreversible under our conditions.

D) Degradation of the DNA-4' radicals

The behaviour of the model radicals is now used to explain the strand break formation and subsequent chemical transformations of the DNA-4' radicals **1** in aqueous solution. Six products have been identified as final products [1, 21, 22], see Table V. The products **23**, **24**, and **25** are base and phosphate free altered sugars, the products **26**, **27**, and **28** are end groups with the same base free altered sugars.

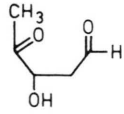
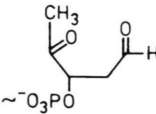
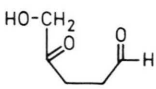
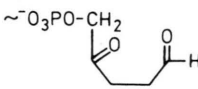
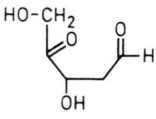
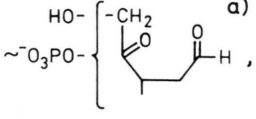
According to the reaction mechanisms outlined above the first step of the strand breaking reaction of **1** is either the elimination* of a 5'-phosphate end group or of a 3'-phosphate end group and the formation of either the 4'-5'-radical cation, **29**, reaction (26), or the 3'-4'-radical cation, **30**, reaction (27), see Scheme 3. These elimination reactions occur spontaneously or proton catalyzed [2].

* An eclipsed conformation is apparently not a principle prerequisite for ring opening of the tetrahydrofuran ring with a radical site at the 3-position. Steenken [20], for instance, showed evidence for a relatively fast ring opening reaction with the radical shown aside. In this case, however, a proton catalyzed water elimination in competition to ring opening is not possible.



* This step might be preceded by a 5'-4' or by a 3'-4' shift of the 5'- or the 3'-phosphate group to yield the isomeric 5'- or 3'-radicals respectively, which subsequently eliminate the phosphate end groups to also yield the radical cations **29** or **30**. This kind of phosphate shift was observed with the model radical **5** with $\text{L} = \text{HPO}_4^-$, prior to hydrolysis [14]. In radical **1** the rate of the phosphate shift may determine the preference for the elimination of the 3'- or the 5'-phosphate group.

Table V. Products identified upon γ -irradiation of N_2O saturated aqueous solutions of DNA and their G -values (number of molecules or end groups formed per 100 eV of energy absorbed by the solution).

Product	G-value	Product	G-value
 , 23	0.05	 , 26	0.025
 , 24	0.01	 , 27	0.07
 , 25	0.06	 , 28	0.05

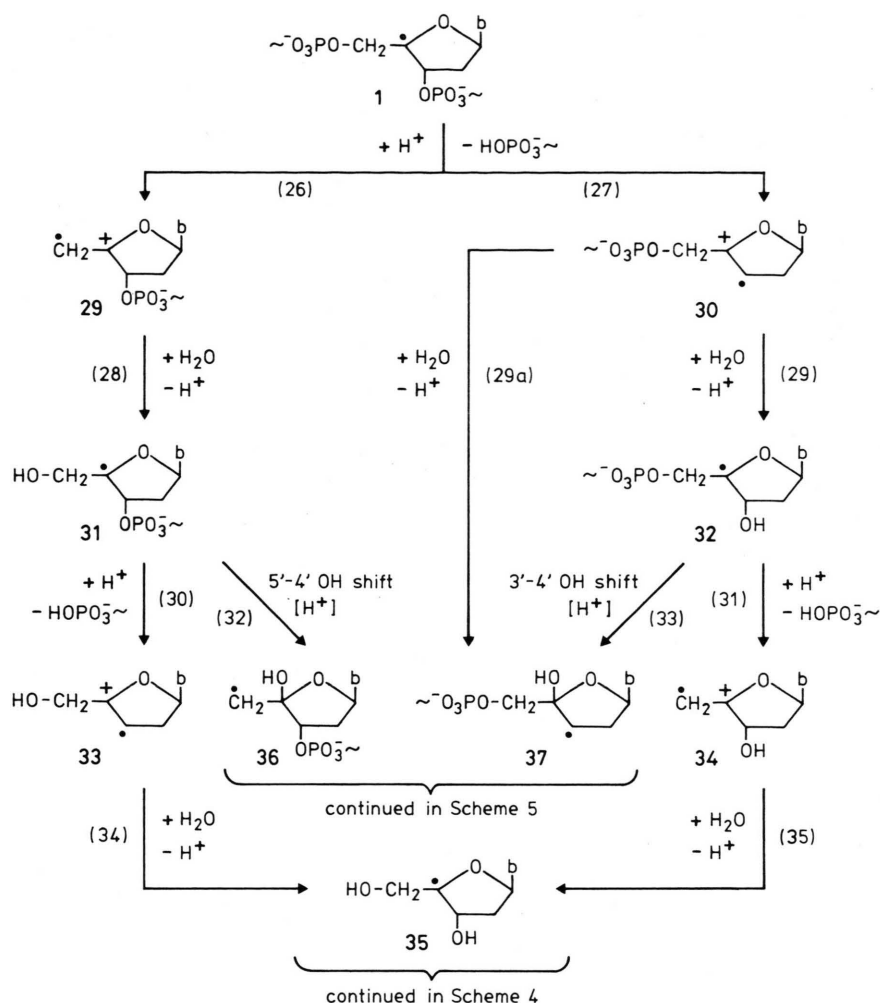
a) Site of OH and remaining strand, \sim_3OPO , is not exactly known.

With the radical cation **29** the ensuing entry of OH^- is at the 5'-position only, reaction (28), as is inferred from the behaviour of the model radicals **8a** or **8b**, reaction (7), Scheme 1. Since steric effects in the hydrolysis reactions of the model radicals, Scheme 1, dominated it is assumed that the entry of OH^- into radical cation **30**, reaction (29), is mainly at the 3'-position with a possible small contribution of reaction (29a). In other terms, it is assumed that the radical **7a** with its two β -positioned leaving groups is a better model for the degradation of **1** than the radicals **9a** or **9b**.

Radicals **31** or **32**, Scheme 3, may now undergo either elimination of the remaining strands, reactions (30) and (31), or proton catalyzed OH shifts, reactions (32) and (33), Scheme 3. Upon elimination of the strand radical cations of low molecular weight, **33** and **34**, are formed which are quite mobile in the aqueous phase. Their reactions with water, (34) and (35), will lead (again assuming regio selectivity) to one common radical, **35**, Scheme 3. This radical is seen as the key radical for the formation of the base and phosphate free altered sugars **23** to **25**, see Table V and Scheme 4. The proton catalyzed OH shifts, reactions (32) and (33) lead to the A-type radicals **36** and **37** which give rise to the base free altered sugars bound to the (broken) strand, **26** to **28**, see Table V and Scheme 5.

For the proton catalyzed OH shifts, reactions (32) and (33), to compete with the elimination reactions (30) and (31), Scheme 3, a relatively high concentration of protons is required. Protons are formed in reactions (28), (29), (34), and (35), Scheme 3. In solutions of DNA protons and metal cations are not homogeneously distributed. It is known [23] that the poly anionic DNA and similar poly anions are surrounded by a layer of counterions in their immediate proximity. For instance, polyuridylic acid in aqueous solution at pH 6–7 has a proton density in the immediate vicinity of the strand that corresponds to a concentration of 0.17 molar [24]. It is by virtue of this that the branching in Scheme 3 with the radicals **31** and **32** appears feasible, since the concentration of protons is increased in the vicinity of the strand in the same proportion as in the solution. Note, that also the elimination reactions (26), (27), (30), and (31) of Scheme 3 are susceptible to proton catalysis, *cf.* [2].

As to the fate of radical **35** in Scheme 4 again proton catalyzed OH shifts are to be taken into account, this time at the pH of the solution since radical **35** is no longer associated with the strand. Now H^+ catalyzed OH shifts have to compete with bimolecular radical-radical termination reactions. With respect to the latter type of reactions the mean radical life under ^{60}Co - γ -irradiation conditions is of



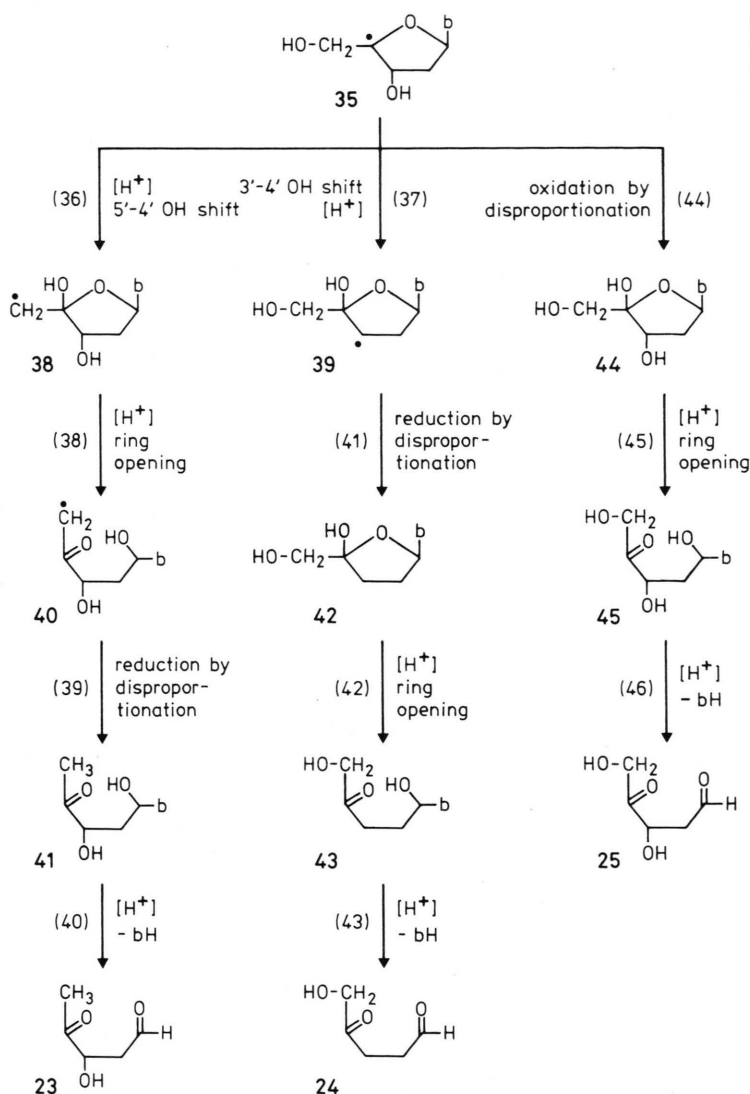
Scheme 3. Degradation of DNA-4' radicals, **1**, in aqueous solution. b = a nucleobase of DNA, ~ = DNA strand continued.

the order of seconds and under our ESR conditions of the order of milliseconds. In view of this difference it is understandable that a reaction which becomes observable at pH 3–4 under ESR conditions is of importance under γ -irradiation conditions already at pH 6–7.

From the model studies it is recognized that either OH group in **35** may undergo an acid catalyzed OH shift, reactions (36) and (37), Scheme 4, yielding the A-type radicals **38** and **39**. Radical **38** will undergo H⁺ catalyzed ring opening to give radical **40**, reaction (38), which on reduction by disproportionation, reaction (39), gives the altered nucleoside **41**, which eventually suffers proton catalyzed loss of its base, reaction (40), Scheme 4, to

yield one of the altered sugars, **23**, observed, Table V. A similar pathway, reactions (41), (42), and (43), Scheme 4, leads to the second of the observed altered sugars, **24**. The difference to the above pathway is that – since the radical **39** cannot undergo ring opening (as is inferred from the model studies) – reduction by disproportionation occurs first, giving **42**, and ring opening occurs thereafter at a non radical stage in a somewhat slower reaction, (42).

A possible pathway to the third altered sugar, **25**, is outlined in reactions (44), (45), and (46) of Scheme 4. In contrast to the ketomethyl radical **40** or the A-type radical **39** the E-type radical **35** may undergo oxidation rather than reduction by disproportionation to give **44** (but see also further below).



Scheme 4. Degradation of DNA-4' radicals in aqueous solution through radical **35**, continued from Scheme 3.

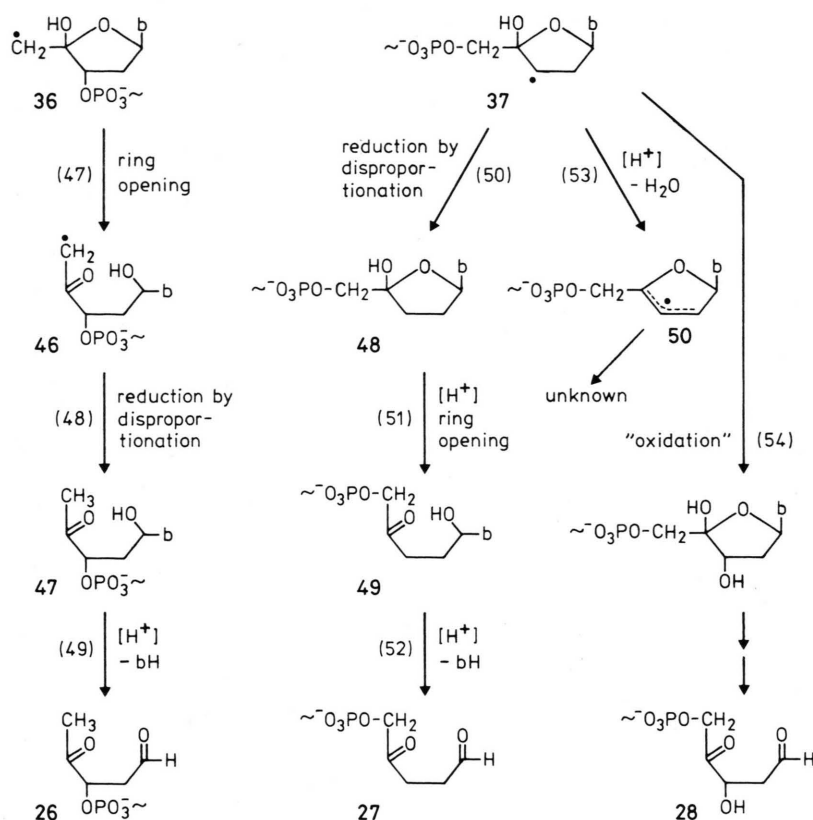
The ensuing ring opening and base elimination reactions, (45) and (46), proceed in a way analogous to the one in the second branch of Scheme 4.

The formation of the altered sugar end groups **26**, **27**, and **28** of Table V via the A-type radicals **36** and **37** is shown in Scheme 5.

Reactions (47), (48), and (49) leading to the altered end group **26** and reactions (50), (51), and (52) leading to the altered end group **27** are analogous to those described in Scheme 4 with the "mobile" intermediates. Since the local acid concentration at the strand is higher than in the bulk of the solution and the lifetimes of the radicals of the

DNA are rather long it appears possible to consider reaction (53) and the formation of an allyl type radical **50**, *cf.* the model reaction (18), Scheme 2. Whether such an allyl radical is formed from DNA is not known.

The mode of formation of the observed third altered end group, **28**, and the location of the phosphate ester linkage in it are not known [1]. Radical **37** and (even less) radical **46** of Scheme 5 are not prone to oxidation by disproportionation reactions – in contrast to radical **35** of Scheme 4. An unknown oxidizing species [1] probably reacts with **37**, reaction (54), Scheme 5, to lead eventually to the



Scheme 5. Degradation of DNA-4' radicals in aqueous solution through radicals **36** and **37**, continued from Scheme 3.

altered end group **28**. Traces of oxygen may be the oxidant. The rigorous exclusion of O_2 as practiced in the present work was not attained in the earlier experiments with DNA. In view of this the formation of **25** in Scheme 4 might well be subject to future revision since, moreover, **25** was recognized [25] as major product after γ -irradiation of aqueous solutions of DNA in the presence of N_2O and O_2 .

The novel features introduced into the Schemes of degradation of the DNA-4' radicals are:

- the regio specific entry of OH at the sites of the eliminated phosphate groups,
- proton catalyzed OH shifts in the radicals in competition with elimination and other reactions,
- ring opening reactions in the radical state before disproportionation, or after disproportionation in a non radical state, depending on the structure of the intermediate radical,
- the possible involvement of the allyl-type radical end group **50** as intermediate and resultant alterations of the strand,

e) (proton catalyzed) phosphate shifts preceding the strand breakage – not given in Scheme 3 but discussed as possibility.

In the earlier Scheme of degradation of **1** [1] suggestions were made on the basis of the product ratios **23**:**24** or **26**:**27** observed (obtainable from the *G*-values given in Table V) as to the preference of elimination of the end group from C-3' of radical **1** over the elimination of the end group at C-5'. In view of the new Schemes it is still certain that either type of strand break, reaction (26) and (27), is involved, as is inferred from the structure of the altered end groups formed, however no conclusions as to the ratio of the two branches can be derived. This is because the preference of formation of **27** over **26**, *cf.* Table V, is not only governed by the first branching, reactions (26): (27), but also by the occurrence of phosphate shifts and by the second branching, reactions (30): (32) and (31): (33), Scheme 3. Estimates of these ratios are presently not available. After elimination of the second leaving group, reac-

tions (30) and (31) the two branches are united in radical **35** and conclusions as to its pathway of formation are no longer possible.

From the discussion of the mechanisms leading to strand breaks it appears that a number of questions remain to be solved. First of all the kinetics of the various steps occurring at the DNA have to be determined which is rather difficult. Further, more detailed knowledge is required concerning the proton concentration at the DNA as a function of the salt concentration in solution. This question will be treated in a forthcoming paper [17].

Experimental

Chemicals

Commercial 1-methoxy-2-propanol was purified by fractional distillation *in vacuo*, from the higher boiling fraction the isomeric 2-methoxy-1-propanol was obtained. 2-Methoxyethyl chloride, 2-methoxyethyl acetate, 2-methoxyethylidene dichloride, 2-hydroxymethyl-tetrahydrofuran and 3-hydroxytetrahydrofuran were commercial samples. The ether-alcohols were converted into ether-chlorides or ether-acetates by standard procedures. 2-Methoxy-1,3-dichloro-propane was obtained from 2-hydroxy-1,3-dichloro-propane by reaction with dimethyl sulfate. All substrates were purified by fractional distillation *in vacuo* to better than 99.8% purity as checked by g.l.c., and stored under argon at 277 K. The structures of the substrates were confirmed by NMR analysis. Water was triply distilled. Other chemicals were of analytical grade.

Oxygen free solutions

For ESR spectroscopy solutions were purged with argon, 99.9999%, Messer-Grießheim, Duisburg. For pulse or γ -irradiation solutions were purged with N_2O , freed from O_2 by passage through columns packed with chromosorb, Messer-Grießheim, Duisburg. All leads and connections carried wide hoses which were purged with argon, $\geq 99.999\%$, to prevent diffusion of O_2 to the solutions through micropores in the leads. With this techniques after γ -radiolysis the results were the same as with the use of multiple freeze-pump-thaw cycles, sealed samples and high vacuum lines.

Radical generation

In pulse radiolysis or ^{60}Co - γ -radiolysis $\cdot OH$ radicals and solvated electrons are formed as main radical species directly from water which was the solvent. N_2O -saturated solutions were used for converting the solvated electrons into $\cdot OH$ radicals, according to $e_{aq}^- + N_2O \rightarrow \cdot OH + OH^- + N_2$. In ESR spectroscopy radicals were formed *in situ* by UV irradiation: $\cdot OH$ radicals from added H_2O_2 , $(CH_3)_3C-O\cdot$ radicals from tert.-butyl peroxide and $SO_4^{\cdot -}$ radical anions from peroxy disulfate, using small amounts of acetone as sensitizer [2]. The substrate concentrations were 10^{-2} M in radiolysis experiments and up to 10^{-1} M for *in situ* ESR measurements.

Analysis of radical structure and product determination

The radicals were identified and their chemical transformations were studied by ESR spectroscopy. Technical details have been described elsewhere [2]. The mean radical life time with respect to bimolecular termination was 100–300 microseconds. Stable chemical products were analyzed after ^{60}Co - γ -irradiation of aqueous solutions of corresponding saturated substrates. The solutions were purged either with N_2O or with N_2O/O_2 mixtures (4:1, v/v) prior to irradiation. H^+ and Cl^- were determined by potentiometric titration and CH_3OH by g.l.c. [2].

Determination of hydrolysis rates

Radical hydrolysis rates were determined by time resolved conductivity measurements in combination with pulse radiolysis [4]. The generation of $\cdot OH$ radicals, *cf.* above, is initiated by a 2.8 MeV electron pulse of 1 microsecond width. The reaction of the $\cdot OH$ radicals with the substrates has half lives of $\approx 10^{-7}$ seconds, thus the formation of the substrate radicals is practically finished at the end of the pulse. Thereafter the formation of acids was followed by measuring the time resolved conductivity increase in solutions with the pH adjusted between 4 and 6. The time resolution of our half life measurements was 1 microsecond. The changes in acid con-

centration per pulse were of the order of a few micromoles per liter. From the magnitude of the changes the yields of radicals undergoing hydrolysis could be determined; they were found to agree with the values derived from titrations after γ -radiolysis.

Acknowledgement

Unremitting technical support of this work by Mssrs. H. Muhlert and H. Niehaus is gratefully acknowledged.

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