

## Spectroscopic Evidence for a Radical Cation as Intermediate in a Model Reaction of the 4'-DNA Radical Strand Cleavage

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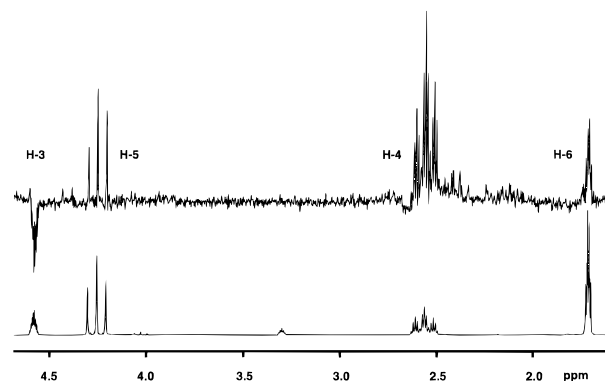
Received May 22, 1997

Revised Manuscript Received July 23, 1997

Radicals are effective neighboring groups to induce nucleophilic substitution reactions.<sup>1</sup> This activation effect might play an important role in the anaerobic scission of 4'-DNA radicals. Whereas ab initio calculations on primary systems make S<sub>N</sub>2-type substitutions feasible,<sup>1a</sup> studies on the radical-induced DNA strand cleavage (**1** → **3**) favor the S<sub>N</sub>1-type reaction with radical cation **2** as intermediate (Scheme 1).<sup>2,3</sup> CIDNP (chemically induced dynamic nuclear polarization) experiments have led to the first spectroscopic proof for the existence of a radical cation as intermediate in a reaction that models the C,O-bond scission of 4'-DNA radicals.

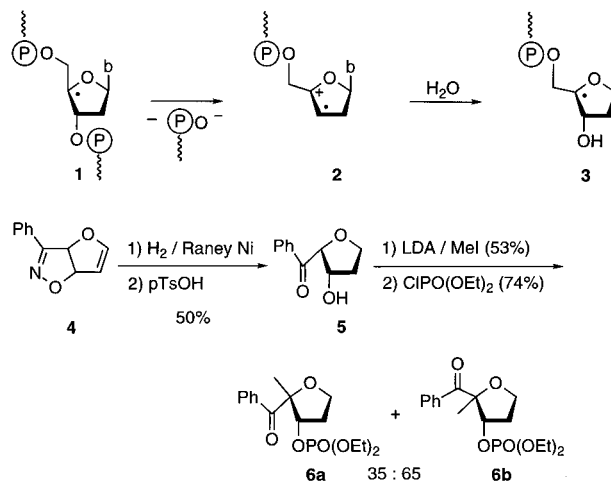
As precursor for these radical reactions, we synthesized ketone **6a** via benzonitrile oxide cycloaddition to furan,<sup>4</sup> subsequent hydrogenation (**4** → **5**),<sup>5</sup> methylation, and phosphorylation. The diastereoisomers could be separated by flash chromatography.<sup>6</sup>

Two different kinds of CIDNP experiments, time-resolved experiments with a maximum time resolution of approximately 50 ns and multiple laser pulse experiments,<sup>7,8</sup> were performed to study the photochemistry of **6a**. In methanol solution, several spin-polarized products could be observed. The major nuclear spin polarizations are due to the enol ether **11**. The resonances recorded in a time-resolved experiment with a resolution of 150 ns are reproduced in Figure 1 and were also observable in the 50 ns experiment but with much smaller signal to noise (S/N) ratio.<sup>9</sup> The chemical shifts and the polarizations of the signals are compiled in Table 1. Chemical analysis proved that **11** is an important product and not solely formed in a side reaction. CIDNP intensities, governed by the radical pair theory, for comparable nuclear spin relaxation times and for hyperfine couplings smaller in magnitude than the absolute value of the



**Figure 1.** Time-resolved CIDNP spectrum (150 ns) of **6a** exhibiting polarized resonances of **11** (upper trace) and reference NMR spectrum of **11** (lower trace).

### Scheme 1



**Table 1.** CIDNP Spectrum (Multiple Laser Pulse Experiment) of Enol Ether **11** Observed by Irradiation of Phenyl Ketone **6a** and Calculated from the Hyperfine Coupling Constants of Table 2

H	chemical shift [ppm]	polarization	rel intensity			relaxation time [s]
			expt	calcd <sup>a</sup>		
3-H	4.55	emission	-5.0	-3.4	(-6.3)	27.9
4-H	2.57	absorption	10.1	8.6	(10.1)	14.5
5-H	4.30	absorption	1.4	2.3	(3.2)	15.2
6-H	1.68	absorption	≡1.0	≡1.0	(≡1.0)	11.7

<sup>a</sup> The numbers in parentheses take the spin-lattice relaxation times into account.

difference in Zeeman energies of the two radicals are approximately proportional to the hyperfine coupling constants.<sup>10</sup> The character of the polarization in such a case is determined by Kaptein's rules.<sup>11</sup> Since the strongest polarization in Figure 1 is observed for the C-4 methylene group, the radical pair after  $\alpha$ -cleavage (**7** + **8**) cannot be responsible for the polarization. However, it might be built up in a pair of benzoyl radical (**7**) and radical cation (**9**) after rapid phosphate elimination (Scheme 2). In order to verify this hypothesis, we calculated the ESR hyperfine coupling constants of the radical cation **9** using three different density functionals known to give reliable results (Table 2).<sup>12</sup>

Alkyl aryl ketones in general undergo  $\alpha$ -cleavage from the

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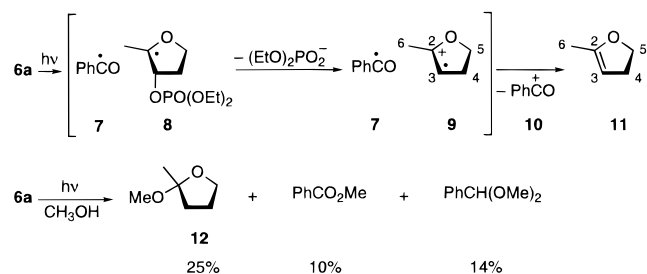
(6) The new compounds were fully characterized by spectroscopic techniques and elemental analysis (data given in the Supporting Information). Compounds **6a** and **6b** (assigned by NOE experiments and *cis*- $\gamma$ -effect) were separated by flash chromatography on silica gel with diethyl ether/ethyl acetate (100:0 to 80:20) as eluents.

(7) For the multiple laser pulse experiment, the observing radiofrequency pulse followed a saturation pulse sequence and 10 laser pulses with delay times of 5 ms.

(8) Phenyl ketone **6a** (28 mg) was dissolved in MeOH-*d*<sub>4</sub> (0.6 mL), degassed for 10 min with N<sub>2</sub>, and sealed in a NMR quartz tube. The solution was irradiated at 25 °C with an excimer laser (Questek) coupled with a dye laser (Lambda Physik) at  $\lambda = 342$  nm (11 mJ/pulse) in the magnetic field of a Bruker AM200 NMR spectrometer (200 MHz wide bore).

(9) The experiment was carried out as in ref 8 using a Nd-Yag laser (Continuum, Surlite II) at  $\lambda = 355$  nm (100 mJ/pulse).

## Scheme 2



**Table 2.** Calculation of ESR Hyperfine Coupling Constants  $a_{\text{H}}$  (mT) of Radical Cation **9** by Three Density Functionals

method	3-H	4-H	5-H	6-H
B3LYP/6-31G*	-1.677	4.276	1.157	0.495
BLYP/6-31G*	-1.489	4.463	1.397	0.564
BPW91/6-31G*	-1.662	4.434	1.437	0.495

first excited triplet state.<sup>13</sup> For compound **6a** this cleavage is accelerated by the oxygen in the five-membered ring. The radical pair theory predicts that cage products (recombination or disproportionation within the pair) carry opposite polarization with respect to their escape counterparts. The observed escape polarization is usually weaker than the cage polarization. The major nuclear spin polarization observed for enol ether **11** therefore strongly points to a formation of this product in a cage reaction. This is further corroborated by the observation of a weak and opposite spin polarization for 2-methylfuran (*vide infra*). As a consequence of precursor state, product formation, and the calculated signs of the hyperfine coupling constants, a benzoyl radical (**7**) has to be involved in the cage reaction to fulfill Kaptein's rule. Its extremely small  $g$ -value leads to the required positive sign for the difference of the  $g$ -values of radicals **9** and **7**.<sup>14</sup>

Under the assumption that the elimination of phosphate (Scheme 2) does not affect the electron spin state and that the enol ether **11** is formed by an electron transfer (SET) from the benzoyl radical **7**, the observed polarizations and intensities are in good agreement with the calculated values (Table 1).

For a more accurate determination of the CIDNP intensities, the spin lattice relaxation times  $T_1$ ,<sup>15</sup> the external magnetic field, and the estimated difference in  $g$ -values of  $2.4 \times 10^{-3}$ <sup>14</sup> were also used (Table 1, values in parentheses).<sup>16</sup> The agreement between the observed and calculated polarizations and the intensities of the CIDNP spectrum is a direct proof for the radical cation **9** as intermediate in this C,O-bond cleavage reaction which undergoes a SET reaction.

Kaptein's rule and the primary  $\alpha$ -cleavage have led to the benzoyl radical as donor in the SET reaction. The calculated gas phase redox potentials of the benzoyl radical **7** and the enol ether **11** are 6.5 and 8.0 eV, respectively.<sup>17</sup> Thus, the electron transfer from benzoyl radical **7** to radical cation **9** is thermo-

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(14) The following  $g$ -values were used:  $g(\mathbf{7}) \approx 2.0006$ ;  $g(\mathbf{9}) \approx 2.0030$  (see (a) Grossi, L.; Placucci, G. *J. Chem. Soc., Chem. Commun.* **1985**, 943. (b) Madelung, O.; Fischer, H. *Landolt-Börnstein New Series*, II/17h, refs 80–84).

(15)  $T_1$  Values were measured by the inversion recovery method on a Varian Gemini 300 (300 MHz) NMR spectrometer.

(16) Borer, A.; Kirchmayer, R.; Rist, G. *Helv. Chim. Acta* **1978**, *61*, 305.

dynamically feasible. This reaction leads to the benzoyl cation **10** which should react with methanol yielding methyl benzoate. Actually, methyl benzoate is formed if phenyl ketone **6a** is irradiated in methanol (Scheme 2).<sup>18</sup> Enol ether **11** could not be detected by GC because it reacts under these acidic conditions (diethylphosphoric acid is liberated) to acetal **12**. The higher yield of acetal **12** compared to that of methyl benzoate is an indication that the trapping of radical cation **9** by methanol competes successfully with the SET reaction ( $\mathbf{7} + \mathbf{9} \rightarrow \mathbf{10} + \mathbf{11}$ ).<sup>19</sup>

As mentioned above, the radical pair between benzoyl radical **7** and radical cation **9** is generated in a cage by scission of the C,O-bond from the first formed triplet pair between benzoyl radical **7** and tetrahydrofuran radical **8**. Thus, this phosphate cleavage ( $\mathbf{8} \rightarrow \mathbf{9}$ ) has to compete (a) with the diffusion of the radicals into the solvent and (b) with the triplet-singlet interconversion. Because of this competition, the observed CIDNP effect demands a very rapid reaction for the cleavage step ( $\mathbf{8} \rightarrow \mathbf{9}$ ). Actually, competition kinetic experiments with an excess of thiophenol as radical trap showed that the heterolysis of the C,O-bond ( $\mathbf{8} \rightarrow \mathbf{9}$ ) is faster than  $3 \times 10^9 \text{ s}^{-1}$ .<sup>20</sup>

With strongly improved S/N ratios such as those in multiple laser pulse experiments,<sup>8</sup> weak resonances of 2-methylfuran were detected carrying escape-type polarization, lending further proof for radical cation **9**. No resonances were observed in the CIDNP experiment for **6a** and its diastereomer **6b**, indicating that phosphate elimination is more effective than recombination to the starting material. However, the aldehyde proton of benzaldehyde (10.0 ppm), formed by hydrogen abstraction, was observed in enhanced absorption.

**Acknowledgment.** This work was supported by the Swiss National Science Foundation. We acknowledge helpful discussions with Dr. T. Winkler, Novartis AG.

**Supporting Information Available:** Spectral data and elemental analysis for compounds **5**, **6a**, and **6b**, including the NOE experiments for **6a** and **6b** (3 pages). See any current masthead page for ordering and Internet access instructions.

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(17) The ionization potentials were calculated with the compound method CBS-Q: Ochterski, J. W.; Peterson, G. A.; Montgomery, J. A. *J. Chem. Phys.* **1996**, *104*, 2598. The calculated ionization potential of 8.0 eV for **11** is in agreement with its photoelectron spectrum.

(18) For the analysis of the products, phenyl ketone **6a** (35 mg) was dissolved in MeOH (1 mL), degassed for 10 min with  $\text{N}_2$ , and sealed in a NMR quartz tube. The solution was irradiated at 25 °C with a Nd-Yag laser (Surelite) at 355 nm (39 mJ/pulse) for 30 min. The products were identified and quantified by GC using undecane as an internal standard and synthesized or commercially available reference substances.

(19) We have not observed CIDNP polarizations for acetal **12**, which is formed by trapping reaction of the radical cation **9** with methanol and subsequent H-abstraction from methanol. The reason is probably the slow H-abstraction step, which is in the order of  $10\text{--}10^2 \text{ M}^{-1} \text{ s}^{-1}$  (see Burchill, C. E.; Ginns, I. S. *Can. J. Chem.* **1970**, *48*, 1232, 2628). Presumably, after this slow reaction step, the polarizations are diminished to such an extent that they are no longer observable for acetal **12**.

(20) The competition kinetic experiment was carried out at 25°C in MeOH with phenyl ketone **6a** (46 mM) and a 10-fold excess of thiophenol (460 mM). After photolysis, the product analysis was carried out by gas chromatography. The product formed by trapping of radical **8** by thiophenol could not be observed. The limit of detection between the stable cleavage product **12** and the H-abstraction product of radical **8** was 200. With this number and an estimated rate of H-abstraction for thiophenol of  $3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  (see Tronche, C.; Martinez, F. N.; Horner, J. H.; Newcomb, M.; Senn, M.; Giese, B. *Tetrahedron Lett.* **1996**, *37*, 5845), the rate for the heterolytic cleavage ( $\mathbf{8} \rightarrow \mathbf{9}$ ) was calculated to be  $>3 \times 10^9 \text{ s}^{-1}$ . With a benzoate instead of a diethyl phosphate as leaving group (**6a**, PhCO instead of PO(OEt)<sub>2</sub>) the intermediate neutral radical analogous to **8** could be trapped, and the C,O-bond cleavage was determined as  $8 \times 10^6 \text{ s}^{-1}$  at 25 °C in MeOH (unpublished results of S. Peukert and M. Obkircher, Basel).