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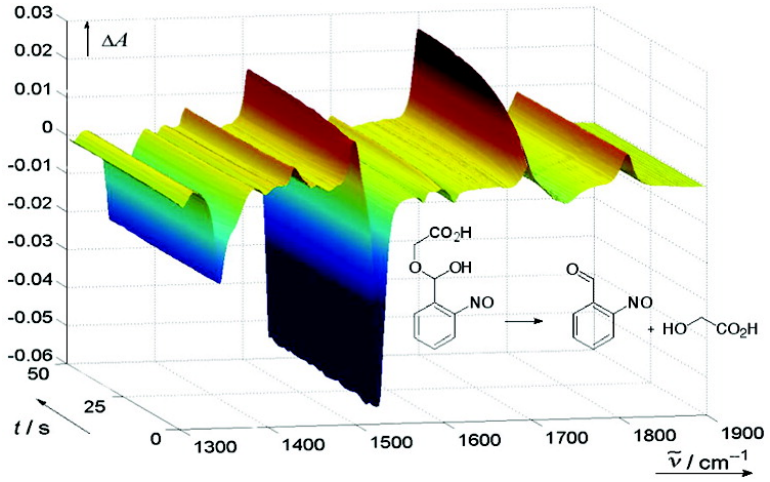

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Photorelease of Alcohols from 2-Nitrobenzyl Ethers Proceeds via Hemiacetals and May Be Further Retarded by Buffers Intercepting the Primary *aci*-Nitro Intermediates

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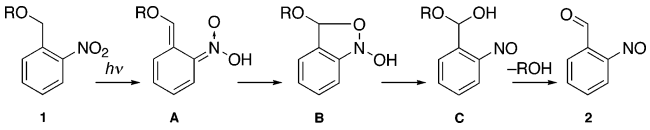
Photoremovable protecting groups are used, inter alia, to determine physiological response times to bioactive compounds. To this end, the release must be faster than the response time of interest.[1] It was recently found that the release of alcohols from 1-(2-nitrophenyl)ethyl ethers proceeds via hemiketal intermediates that, under physiological conditions, have lifetimes around 10 s.[2,3] Therefore, they cannot be used for time-resolved work.

For 2-nitrobenzyl ethers **1**, the reaction via the formation of a hemiacetal intermediate **C** (Scheme 1) was explicitly excluded in an investigation of the glycolic acid ether **1** (R = CH_2CO_2H).[2] Yet, we identified[3] the decay of **C** as the rate-limiting step for the release of methanol from the methyl ether (**1**, R = Me) in aqueous solutions, pH < 8. Here, we show that the release of glycolic acid from **1** (R = CH_2CO_2H) also proceeds via a hemiacetal **C** and, moreover, that buffers can trap the primary *aci*-intermediates **A**, thereby further retarding alcohol release.

Scheme 1



Intermediates of type **A**, **B**, and **C** had been observed by nanosecond laser flash photolysis (248 nm, 25 ns) of **1** (R = Me) in aqueous solution.[3] Quite similar results were obtained here with **1** (R = CH_2CO_2H), Table S1.[4] In the pH range of 2−6, the formation of 2-nitrosobenzaldehyde (**2**) was sufficiently slow to be monitored on a conventional spectrophotometer. The reaction is associated with a characteristic[3] increase in absorbance at 236 nm (Figure S1).[4] Surprisingly, these absorbance changes were best fitted by a biexponential rate law. In contrast, the reaction with R = Me had obeyed a first-order rate law with rate constants close to those of the faster component, k_1, of the glycolic acid ether reaction. The second component, k_2, is an order of magnitude slower. The ratio of the two 236-nm amplitudes, $A(k_1)/A(k_2)$, depends strongly on pH (Table S2 and Figure S2).[4] It is minimal at pH 3−4, which indicates that the reaction via the longer-lived intermediate predominates in this pH range.

Time-resolved infrared (TRIR) experiments provide direct evidence for the presence of functional groups (−C═O, −N═O) in the observed reactive intermediates. A solution of **1** (R = CH_2CO_2H, ca. 20 mM) in CD_3CN containing 20% D_2O and 0.01 M DCl in a CaF_2 cell of 200 μm path length was irradiated for 3 s with the frequency-quadrupled output of a Nd:YAG laser (266 nm, 10 Hz, 5 mJ/pulse). IR spectra (scan duration 70 ms) were then recorded repeatedly for 60 s. The resulting difference spectra (spectrum at delay t − spectrum prior to irradiation) were subjected to factor analysis. The spectra were reproduced, within experimental error, by linear combination of the two major spectral components, providing a reduced set of data. The rate constant obtained by global

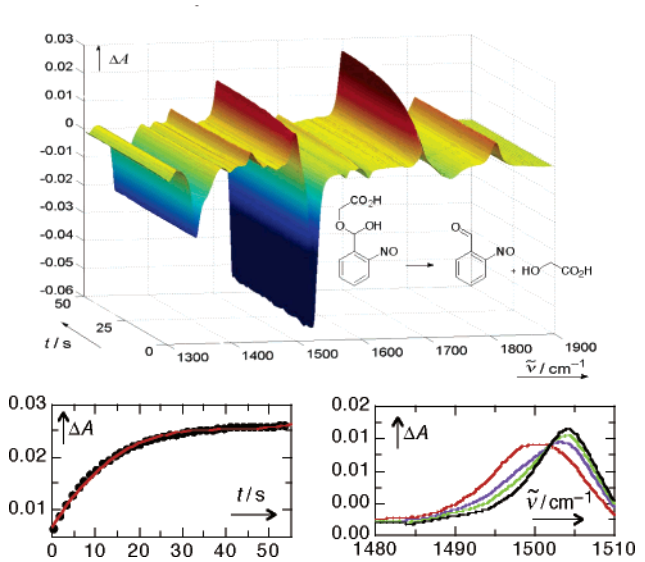


Figure 1. TRIR spectra of **1** in CD_3CN with 20% D_2O and 0.01 M DCl. The lower panels show a kinetic trace of the growth of the 1698-cm^{-1} band (left) and a detailed view of the shift of the nitroso stretching vibration from 1500 to 1505 cm^{-1} during the observation period (right).

least-squares fitting of a first-order rate law to the reduced data (Figure 1), $k = (8.5 \pm 0.2) \times 10^{-2}$ s^{-1} (35 °C), is in satisfactory agreement with that determined by the absorbance growth at 236 nm in the same, but nondeuterated solvent mixture, $k_1 \approx 5 \times 10^{-2}$ s^{-1} (25 °C). The lower panels of Figure 1 display a kinetic trace for the appearance of a C═O band at 1698 cm^{-1} (left) and the concomitant shift of the N═O band from initially 1500 to 1505 cm^{-1} (right). These observations leave no doubt that the faster process observed by UV and IR is the reaction **C** → **2** + ROH (R = CH_2CO_2H).

In addition to the variable features in the sequence of spectra (Figure 1), a positive band at 1809 cm^{-1} is present throughout the 60 s covered by the scans. Thus, another compound, which is stable for at least 60 s, is formed in addition to **C** by the laser flash. The yield of the new compound was higher in a solution containing more water but no acid (75% D_2O, 25% CD_3CN, ca. 2 mM of **1**). Here, the depletion of absorbance in the region of the free carboxyl group of **1** (1730 cm^{-1}) and the intensity of the positive band at 1809 cm^{-1} were much more pronounced, while the growth amplitude of the 1698-cm^{-1} C═O band of **2**, $k = (4.1 \pm 0.6) \times 10^{-2}$ s^{-1}, was substantially reduced.

The position of the 1809-cm^{-1} band suggested that the new compound might be a dioxolanone.[5] To obtain NMR spectra of this product, a solution of **1** (R = CH_2CO_2H, 10 mg) in 0.7 mL of CD_3CN/D_2O (1:1 by vol.) was irradiated for 15 min in a quartz NMR tube with the frequency-quadrupled output of a Nd:YAG laser (266 nm, 10 Hz, 5 mJ/pulse). Irradiation produced new signals that

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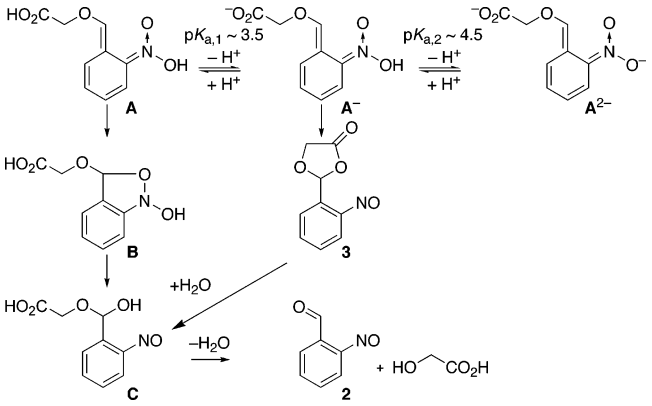
Scheme 2. Thermal Reactions of the Primary *aci*-Tautomers (only the *E* Isomer Is Shown) Formed by Irradiation of **1** (R = CH_2CO_2H)



belong to three different compounds, two of them being the expected 2-nitrosobenzaldehyde (**2**) and glycolic acid, which were identified by comparison with the ^{1}H NMR spectra of authentic samples in the same solvent mixture. The new compound was identified as 2-(2′-nitrosophenyl)-1,3-dioxolan-4-one (**3**) on the basis of its NMR spectral data.[4] These spectra exhibited all features characteristic for an ortho-substituted nitrosobenzene,[5,6] especially the unusual upfield shifts of C_3 and H_3. Most of the heteronuclear correlations expected for **3** were also detected.

To determine the stability of **3**, another solution of **1** (R = CH_2CO_2H, 5 mg) in 0.7 mL of CD_3CN/D_2O (4:6 by vol.) with no added acid was irradiated for 15 min with the Nd:YAG laser, and ^{1}H NMR spectra were recorded 15, 250, 500, and 1400 min after irradiation. The decay of the CH_2 and CH signals of **3** at δ 4.62 and 8.19 ppm, respectively, as well as the concomitant growth of the CH_2 signal due to released glycolic acid, δ 4.07 ppm, all obeyed a first-order rate law, $k = (1.0 \pm 0.1) \times 10^{-4}$ s^{-1}. 2-Nitrosobenzaldehyde (**2**) is not quite stable under the reaction conditions: the NMR signals of **2** exhibited growth, $k_{growth} \approx 1.1 \times 10^{-4}$ s^{-1}, followed by decay, $k_{decay} \approx 3 \times 10^{-5}$ s^{-1}.

The difference between the rate constants for the reaction **3** → **2** determined by H NMR (40% CD_3CN), $k = (1.0 \pm 0.1) \times 10^{-4}$ s^{-1}, and that determined by absorption spectroscopy in wholly aqueous solution, $k_2 = 6 \times 10^{-3}$ s^{-1}, is due to the cosolvent. The optical measurements showed that the reaction is strongly retarded by addition of acetonitrile to water, $k_2 = 2 \times 10^{-3}$ s^{-1} (10%) and $k_2 < 1 \times 10^{-3}$ s^{-1} (20% CH_3CN). The hydrolysis of **3** requires formation of an ionic intermediate and is strongly inhibited by even small amounts of acetonitrile.

Dioxolanone **3** was a minor product in strongly acidic and in neutral aqueous solutions (Table S1 and Fig. S2), but dominated at the expense of intermediate **C** in the absence of added acid or buffer. Such solutions were still slightly acidic, pH ≈ 3.2, due to the presence of 2 mM of the carboxylic acid **1** (R = CH_2CO_2H). Cyclization to form **3** should be most favorable from the monoanion of the *aci*-intermediate $\mathbf{A}^-$, as shown in Scheme 2. The concentration of $\mathbf{A}^-$ is expected to peak around pH 4, because the pK_a of the carboxylic acid function should be about 3.5,[7] that of the nitronic acid function about 4.5.[3] Indeed, the relative amount of **3** is maximal in the pH range of 3−4 (Table S1).[4] The lifetime of the hemiacetal **C** is about 10 s at pH < 6 and low buffer concentrations, as expected from previous work.[3]

Buffer concentrations were kept low (≤0.03 M). At high buffer concentrations the yield of **C** is reduced. For example, an increase of the acetic acid buffer concentration to 1 M (buffer ratio 1:1) reduced the amplitude of the reaction **C** → **2** by a factor of 4. This indicates that the free acetate also adds to the benzylic position of the *aci*-nitro intermediate **A**. The resulting addition product was not identified, but is expected to have a lifetime similar to that of **3**, i.e., trapping of **A** by the buffer is also likely to delay the release of the alcohol.

We have shown that the nitroso intermediates **3** and **C** are formed in dilute, wholly aqueous solutions and that they hydrolyze with rate constants of about 6×10^{-3} s^{-1} and 0.25 s^{-1}, respectively (pH = 7, 20 °C). Why, then, were NO bands not detected in the previous TRIR investigation of the same compound,[2] leading the authors to exclude reaction via hemiacetal **C**?

As the pH of aqueous solutions approaches 7, the slow UV-spectral changes observed after irradiation are no longer consistent with a clean formation of **2**. Slightly above pH 7 the absorption spectra become featureless and GC−MS analysis of the irradiated solution indicates a highly complex mixture containing products of high molecular weight. The previous time-resolved IR study of **1** (R = CH_2CO_2H)[2] was done at pH 8.5 and with a high buffer concentration (200 mM bicine). Therefore, the nitroso compounds **3** and **C** did not accumulate under the reaction conditions. Remarkably, the same authors did observe an absorbance rise at 740 nm (pH 7, 20 °C, $k = 590$ s^{-1}), which they attributed to formation of an aromatic nitroso compound.

In summary, the hemiacetal **C** is formed from 2-nitrobenzyl ethers **1** (R = CH_2CO_2H or Me), and hydrolysis of **C** does limit the release rate of the corresponding alcohols in wholly aqueous solutions at pH values ≤7. In view of these results it is surprising that photolysis of 2-nitrobenzyl-caged D-glucose (**1**, R = D-glc) induced chemotaxis of *Escherichia coli* bacteria with response rates as fast as 15 s^{-1}.[8,9] A finding that may well be relevant to nitrobenzyl-protected compounds, in general, is that interception of the *aci*-intermediates **A** by buffers may further retard release of the desired alcohol. The formation of dioxolanone **3** with a lifetime of 3 min in wholly aqueous solution is an intramolecular example for the trapping of **A** by a "buffer", providing structural proof of concept.

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Supporting Information Available: NMR spectral data of **3** and kinetic data for the intermediates **A**−**C** formed by excitation of **1** (R = CH_2CO_2H). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Pellicioli, A.-P.; Wirz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441−458. (b) Goeldner, M., Givens, R. S., Eds. *Dynamic Studies in Biology*; Wiley-VCH: Weinheim, 2005.
(2) Corrie, J. E. T.; Barth, A.; Munasinghe, V. R. N.; Trentham, D. R.; Hutter, M. C. *J. Am. Chem. Soc.* **2003**, *125*, 8546−8554.
(3) Il'ichev, Y. V.; Schwörer, M. A.; Wirz. J. *J. Am. Chem. Soc.* **2004**, *126*, 4581−4595.
(4) Supporting Information.
(5) Pearson, W. H.; Cheng, M.-C. *J. Org. Chem.* **1987**, *52*, 1353−1355.
(6) Gowenlock, B. G.; Cameron M. *Can. J. Chem.* **1994**, *72*, 514−518.
(7) The pK_a (I = 0.1 M, 25 °C) of methoxyacetic acid is 3.5: Headley, A. D.; Starnes, S. D.; Wilson, L. Y.; Famini, F. G. *J. Org. Chem.* **1994**, *59*, 8040−8046.
(8) Lux, R.; Munasinghe, V. R. N.; Castellano, F.; Lengeler, J. W.; Corrie, J. E. T.; Khan, S. *Mol. Biol. Cell* **1999**, *10*, 1133−1146.
(9) A fraction of the glucose might be released directly from intermediate **B**, **B** → **2** + D-glc. This shortcut could be favored by the steric bulk of D-glc. Whether alcohol release from 2-nitroveratryl ethers also proceeds via long-lived hemiacetal intermediates is not known, but the frequently adopted assumption that the release rate is equal to the decay rate of the readily observable *aci*-nitro transients is clearly not safe at this point.

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