Photoprocesses of Molecules with 2-Nitrobenzyl Protecting Groups and Caged Organic Acids

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ABSTRACT

The 308 nm photoinduced formation of the nitroso product and the intermediacy of the aci-nitro form(s) were studied for a series of 2-nitrobenzyl alkyl and aryl esters (1a-4e) and bis-(nitrophenyl)methyl acetates (5a-6b) by time-resolved UV-vis spectroscopy. A triplet state appears as major transient, when 2-nitrobenzyl derivatives 1 are substituted by 4,5-dimethoxy (2) and 4,5-methylenedioxy (3/4) groups. This triplet of charge transfer character is, however, not part of the route via the acinitro into the 2-nitroso form. The activation energy and preexponential factor of the longest lifetime component (τ_{aci}), i.e. the major part of the aci-nitro decay, were determined. The carboxylic acids as leaving groups have rather small effects on τ_{aci} . An additional nitrated phenyl ring in α -position (5) leads generally to shorter τ_{aci} value. Otherwise, the photogeneration of nitroso products is similar. The quantum yield (Φ_d) varies only moderately with structure, the yield of the *aci*-nitro form and Φ_d are correlated and little affected by solvent properties.

INTRODUCTION

2-Nitrobenzyl-*caged* compounds have become important as the irreversible photoreaction of the protecting group can be used to trigger the release of an appropriate biochemically active substrate (1–7). A large number of photolabile protecting groups with the 2-nitrobenzyl function have been developed that can release bioactive compounds rapidly upon photolysis with UV/visible light (8). The photoreaction is triggered by intramolecular benzylic H-atom transfer to the excited nitro group. Efficient formation of the *aci*-nitro form is known for several cases (5,9–15).

The photoreaction of 2-nitrobenzyl (HXCRArNO₂ in Scheme 1) into 2-nitroso compounds has been extensively studied (16–28). 2-Nitrosobenzenes (OCRArNO) are the products and the *aci*-nitro form (denoted as HA/A⁻) is the major intermediate in this photoreaction, whereas the cyclic benzisoxazoline (HB/B⁻) and the hemiacylal (HC/C⁻) has not been observed in most cases. The *aci*-nitro form is characterized by an absorption maximum (λ_{aci}) at 400–440 nm and a lifetime (inverse rate constant: τ_{aci}) in the μ s-ms range. Eventually, the leaving group (HX) is released. For different α -substituted *N*-(2-nitrobenzyl)ureas (R: H, Me, carboxy) urea release occurs with quantum yields of 0.4–0.8; $\lambda_{aci} = 440 \text{ nm}$ and $\tau_{aci} = 10 \ \mu \text{s}$ for R: carboxy (15). Radicals, observed upon photolysis of different 2-nitrobenzyl-caged-ATP derivatives in the 1 s range, were proposed to be generated by electron transfer from the *aci*-nitro anion (A⁻) to the nonexcited substrate (16).

The photochemical properties of 2-nitrobenzyl acetate (1a) and several bis-nitrophenyl acetates were studied (12). Photolysis of 2-nitrobenzyl benzoate (1b) yields the *aci*-nitro form as intermediate and 2-nitrosobenzaldehyde and benzoic acid as products with $\Phi = 0.08$ (6). Excited 4,5-dimethoxy-2-nitrobenzyl acetate (2a) has been used as electron donor to photoreduce cytochrome C (7). Recently, for 2-nitrobenzyl alcohol and 1-(2-nitrophenyl)ethanol a novel pathway *via* the nitroso hydrate without cyclization has been established (21). This mechanism should not contribute to the photoreaction observed, because here the transfer of an acylium cation instead of a proton would be necessary. The knowledge concerning 2-nitrobenzyl ethers and carboxylates is still rather limited. It seemed therefore desirable to obtain a deeper insight into the photoinduced reaction of the 2-nitrobenzyl moiety (Chart 1).

This article aims at the photoreactions of 2-nitrobenzylbased derivatives into 2-nitroso compounds and the leaving group HX, which is acetic acid for the acetates (a: 1, 2, 3 and 4), benzoic acid for the benzoates (b) and 4-toluic acid for the toluates (c). The effects of 4,5-dimethoxy (2) and 4,5-methylenedioxy (3,4) substituents on the photoinduced generation of the aci-nitro and the nitroso forms were examined, whereby 2-(4-aminobutyryloxy)-2-(4,5-methylenedioxy-1-nitrophen-2-yl) acetic acid (4d) and 2-(4-aminoglutaryloxy)-2-(4,5-methylenedioxy-1-nitrophen-2-yl)acetic acid (4e) contain amino acid building blocks, which are known to be important neurotransmitters. Moreover, bis-nitrophenyl acetates (5, 6) were studied. For biological application the chromophore of the protecting group should absorb at wavelengths above 300 nm, such that the bioactive material is not photochemically damaged. Therefore compounds of type 2-4 were designed to have absorption maxima at 340-360 nm.

MATERIALS AND METHODS

The synthesis of a 2-nitrobenzyl acetate containing a rigid amino group (7) and the other 18 compounds is described in detail elsewhere (27). The sample purity was ascertained by HPLC analyses, for which a 125×4.6 mm Inertsil C₁₈ 5 μ m column was used and MeOH-water (2:1, vol) as eluent, flow rate: 0.8 mL min⁻¹. The solvents, *e.g.*

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Scheme 1.



Chart 1. 1: 2-nitrobenzyl compounds, 2: 4,5-dimethoxynitrobenzyl compounds, 3: 4,5-methylenedioxynitrobenzyl compounds, 4: α -carboxy-4,5-methylenedioxynitrobenzyl compounds; (a) acetic acid esters, (b) benzoic acid esters and (c) toluic acid esters.

dimethylsulfoxide: DMSO (Fisher), ethanol and acetonitrile (Merck, Uvasol), were used as received; water was purified by a milliQ system. The molar absorption coefficients of **1a**, **1b** and **1c** in acetonitrile at 254 nm are $\varepsilon_{254} = 0.8 \times 10^4$, 1.0×10^4 and 1.4×10^4 m⁻¹ cm⁻¹, respectively. The long-wavelength band of **2a–3c** has typically

 $\varepsilon_{335}=0.6\times 10^4~\text{m}^{-1}~\text{cm}^{-1}$. The UV–Vis absorption spectra were monitored on two spectrophotometers, a diode array (Hewlett Packard, 8453) and one from Perkin Elmer (Lambda 19). For photoconversion the 254 nm line of an Hg lamp was utilized, absorbances of 0.8–2.5 were used for $\lambda_{\rm irr}=254$ nm. The quantum

yield of decomposition Φ_d was determined using optically matched solutions $(A_{254} = 1.5-2)$ and the uridine/water/oxygen actinometer (29). For a few cases, e.g. in DMSO, the 313 nm line of a 1000 W Hg lamp, a monochromator and the aberchrome 540 actinometer were used (30). The retention times under our conditions were 3.0, 8.8, 14.0, 2.8, 8.4, 12.6, 3.2, 10.6 and 16.2 min for 1a-3c, respectively, 10.0 min for 4b and 10.4 min for 5a. An excimer laser (Lambda Physik, Göttingen, Germany; EMG 200, pulse width of 20 ns and energy <100 mJ) was used for excitation at 308 nm. The absorption signals were measured with two digitizers (Tektronix 7912AD and 390AD). Absorbances of 0.3–1.5 at λ_{exc} were used, corresponding to [1a] of 0.1– 0.5 mm. The $\Delta A_{\rm T}$, values were measured with respect to $\Delta A_{\rm T}^{\rm max} = 1$ ($\varepsilon_{520} \times \Phi_{\rm isc} = 6 \times 10^3 \text{ m}^{-1} \text{ cm}^{-1}$) for the triplet state of benzophenone in acetonitrile. The respective experimental errors of E_a and Φ_d are ± 8 and $\pm 12\%$ and those of $\tau_{\rm T}$ and $\tau_{\rm aci}$ are typically $\pm 20\%$. Phosphorescence of singlet molecular oxygen at 1269 nm was detected as described previously (31). Oxygen concentrations of 2.2, 9.1 and 1.3 mm were used for saturation values in DMSO, acetonitrile and water, respectively. The quantum yield (Φ_{Δ}) of formation of $O_2({}^{1}\Delta_g)$ was obtained using optically matched solutions ($A_{308} = 0.8$) and phenazine as reference $\Phi_{\Delta}^{\text{ref}} = 0.9$ (32).

RESULTS AND DISCUSSION

A major aim of this study was to better understand the function-structure relationship concerning the formation and decay of aci-nitro forms (HA/A⁻) and the release of the leaving group. HX is acetic acid (a: 1-4), benzoic acid (b: 1-4) and methyl-benzoic acid (c: 1-4). The effects of 4.5-dimethoxy (2) and 4,5-methylenedioxy (3,4) groups and an additional nitrophenyl ring (5,6) were examined. A classification may be the following: Compounds of group 1 have no 4,5-substituents, of groups 2/3 have 4,5-dimethoxy and 4,5-methylenedioxy substituents and of group 4 are similar, but contain a carboxy group and for 4d and 4e additionally an amino acid building block. Two nitro groups without and with a carboxy group constitute compounds of groups 5 and 6, respectively. All 2-nitrobenzyl-caged compounds follow a general pattern except for a 2-nitrobenzyl acetate 7 which was found to be photochemically inert. This is almost to be expected for substitution by an amino group.

Transients



The transient absorption spectrum of 2-nitrobenzyl benzoate **1b** in dichloromethane (Fig. 1b), DMSO, acetonitrile or

Figure 1. Transient absorption spectra of (a) **1a** in acetonitrile and (b) **1b** in dichloromethane at 20 ns (\bigcirc) , 1 μ s (\triangle) , 0.1 ms (\bigcirc) , 1 ms (\triangle) and 10 ms (\bigcirc) after the 308 nm pulse; insets: kinetics at 400 nm.

ethanol at the end of the 308 nm pulse has a maximum at $\lambda_{aci} = 410$ nm. The decay follows essentially first-order kinetics (rate constant: $1/\tau_{aci}$) with two components or one, depending on the solvent. The spectra of the acetic acid 1a and toluic acid 1c esters in acetonitrile (Fig. 1a) are similar. For 1a-1c in dichloromethane and water-free polar solvents the decay kinetics could not be fitted mono-exponentially in most cases, but the major decay is the longer lived component with τ_{aci} in the 1–10 ms range. A triplet nature is excluded on the basis that triplets of unsubstituted 2-nitrobenzyl compounds have sub-ns lifetimes (1) and oxygen does not affect the decay. The transient is therefore assigned to the aci-nitro structure. The two components of the aci-nitro forms of 1c in acetonitrile have τ_{aci} values of 5 ms (70%) and 0.05 ms and deprotonation of the aci-nitro form into A⁻ has been excluded for 1b in acetonitrile by conductivity studies (6). A biphasic decay with τ_{aci} in the 0.01–0.1 s range was also found for α -carboxy-4,5-methylenedioxynitrobenzyl compounds 4a-e in DMSO/water (Table 1).

On the other hand, when water was added to DMSO, acetonitrile or ethanol, a faster decay at $\lambda_{aci} = 410$ nm and a grow-in due to conversion into a secondary transient with maximum at 500 nm was detected. The conversion into the 500 nm transient is shown in Fig. 2a for **1b** in wet DMSO.

Table 1. Lifetime τ_{aci} (ms) of the longer lived *aci*-nitro transient.*

Comp.	CH_2Cl_2	MeCN	DMSO	DMSO-H ₂ O	EtOH
1a	3	2	3	1	14
1b	0.03/6	0.01/4	<1/7	4	14
1c	5	0.03/3	6	4	8
3b			50		
4a		30	60	5/50	140
4b		30	90	5/60	100
4c		40	100	5/80	160
4d			>90	5/>50	12
4 e			> 50	3/>50	
5a	0.015	0.02	0.03	0.01	0.012
5b	0.01	0.02	0.03	0.01	0.016
6a†		0.001/50	0.001/200) 3	1.2
6b			0.2/3		5

*At 25°C using $\lambda_{exc} = 308$ nm, two transients as indicated. †See Figs. 7 and 8 and text.



Figure 2. Transient absorption spectra of **1b** in (a) DMSO-H₂O (10:1) and (b) DMSO-H₂O (1:1) at 20 ns (\bigcirc) , 10 μ s (\Box) , 0.1 ms (\spadesuit) , 1 ms (\spadesuit) and 10 ms (\blacksquare) after the 308 nm pulse; insets: kinetics at 400 nm (left) and 480 nm (right).

Addition of 50% water does not significantly change the yield, but redshifts λ_{aci} after a few μs and decreases the lifetime (Fig. 2b). A redshift of λ_{aci} within a few microseconds, due to deprotonation of the aci-nitro form into A⁻, has been reported for 2-nitrophenylethyl acetate-caged compounds in a 1:1 acetonitrile-water mixture (22). The presence of water in polar solvents should favor the deprotonation. The two components of the aci-nitro forms of 1a in DMSO-phosphate buffer (1:1) each contribute 50% and have τ_{aci} values of 0.05 and 0.36 ms, which is in good agreement with earlier results of 0.07 and 0.65 ms (68%) in water (25). Formation of the aci-nitro form with similar properties occurs gradually also for 1a and for 1c (Table 1). In this work, the curves for several compoundsolvent combinations were fitted by one or two first-order components. For 1b in acetonitrile the first-order fits were appropriate for analysis of the data presented, but at higher laser intensity, e.g. larger than 1 MWcm⁻² or 25 mJ per pulse (which was avoided), a mixed first- and second-order decay was found. Inspired by the results for 2-nitrobenzyl alcohol in hexane, where a deprotonation of the aci-nitro form was attributed to a molecule of the starting material (21), we take this idea a step further and propose that a second-order process is due to an encounter of two aci-nitro forms, reminiscent to the case of T-T annihilation. Such a reaction with a proton transfer catalyst can, in principle, take place in any solvent.

The transient absorption spectra of 2a-3c in acetonitrile are much broader and the decay is rather short-lived (Fig. 3a,b and Table 2). The major difference is the effect of quenching by oxygen. Similar results were found in dichloromethane, chloroform, DMSO or ethanol. Therefore we propose to assign this transient to a triplet state. This assignment is supported by the observed formation of singlet oxygen. The lifetime (inverse first-order rate constant: τ_{T}) in these solvents and DMSO-water is in the 0.4–2 μ s range, *i.e.* much shorter than typical τ_{aci} values. As accompanying weak signal the longer lived aci-nitro form was also obtained. The ratio of absorbances of the triplet state at the pulse end $(\Delta A_{\rm T})$ and the *aci*-nitro form at a few μ s (ΔA_{aci}) is not changed by the presence or absence of oxygen, *i.e.* the triplet state is not the precursor of the aci-nitro form. The alternative that triplet quenching by oxygen also populates the aci-nitro form is rather unlikely. The rate constant for triplet quenching of 2a-3c in acetonitrile by oxygen is $k_{\rm ox} = (0.9-1.5) \times 10^9 \text{ m}^{-1} \text{ s}^{-1}$, *i.e.* slightly below the diffusion-controlled limit.

To illustrate characteristic examples, transient absorption spectra and typical kinetics are shown in Figs. 4-7. For



Figure 3. Transient absorption spectra in acetonitrile (under air) of (a) **2b** and (b) **3a** at 20 ns (\bigcirc), 1 μ s (\triangle) and 10 μ s (\square) after the 308 nm pulse; insets: kinetics at 500 nm (argon-, air- and oxygen-saturated: upper to lower, respectively).



Figure 4. Transient absorption spectra of **4b** in argon-saturated (a) acetonitrile and (b) DMSO-H₂O (1:1, pH 4) at 20 ns (\bigcirc), 10 μ s (\square), 10 ms (\blacksquare) and 100 ms (\blacklozenge) after the 308 nm pulse; insets: kinetics at 400 nm (upper) and 500 nm (lower).

Table 2. Lifetime (μ s), relative efficiency of the triplet transient and quantum yields of O₂($^{l}\Delta_{g}$) formation.*

Comp.	$\tau_T \ CH_2 Cl_2$	$\tau_{\rm T}$ DMSO	$\tau_{\rm T}$ MeCN	$\tau_T \text{ DMSO-H}_2\text{O}\dagger$	ΔA_{T} ‡	Φ_{Δ} CH ₂ Cl ₂
2a	0.7 (0.3)	0.7 (0.3)	2 (0.5)	0.3	0.8	0.5 [0.2]
2b	1.0 (0.2)	0.8 (0.3)	1 (0.3)	0.4	0.95	0.4 [0.2]
2c	1.1(0.3)	0.7(0.3)	2(0.3)	0.7	0.9	0.4 [0.2]
3a	0.4(0.2)	1 (0.4)	2(0.3)	0.4	0.9	0.3 [0.1]
3b	0.4(0.2)	1 (0.4)	3 (0.3)	0.5	0.95	0.3 [0.1]
3c	0.4(0.2)	1 (0.4)	2(0.3)	0.4	1	0.5 [0.2]
4a		0.6 (0.3)	3 (0.3)		0.3	< 0.1
4b		0.8 (0.3)	3 (0.3)	>2	0.3	
4c		0.6 (0.3)	2	>2	0.4	
4d		1	1	1	0.3	
4e		1		1	0.3	

*Using $\lambda_{exc} = 308 \text{ nm}$, τ_T refers to argon-saturated solution. †1:1 mixture. ‡Relative value in DMSO at 440 nm and the pulse end; similar values for **2a–4c** in dichloromethane, acetonitrile and DMSO-H₂O (1:1). §Values outside and within brackets refer to oxygen- and air-saturated solution, respectively. ||Values in parentheses refer to oxygen-saturated solution.



Figure 5. Transient absorption spectra of **4d** in (a) DMSO and (b) DMSO-H₂O (1:1, pH 4) at 20 ns (\bigcirc), 10 μ s (\square), 10 ms (\blacksquare) and 100 ms (\blacklozenge) after the 308 nm pulse; insets: kinetics at (a) 400/500 nm and (b) 420 nm.



Figure 6. Transient absorption spectra of **5b** in (a) DMSO and (b) DMSO-H₂O (1:1, pH 7) at 20 ns (\bigcirc), 1 μ s (\triangle), 10 μ s (\square) and 0.1 ms (\bullet) after the 308 nm pulse; insets: kinetics at (a) 450 nm and (b) 340 and 420 nm.

 α -carboxy-4,5-methylenedioxynitrobenzyl compounds 4a-c (Fig. 4) as well as for 4d (Fig. 5) and 4e the triplet state appears as a major short-lived transient. For the acetic acid ester 4a in DMSO with $\tau_T = 0.6 \ \mu s$ the shoulder in T-T absorption is replaced by a maximum at 530 nm. Observation of the phosphorescence of singlet molecular oxygen, $O_2(^{1}\Delta_{g})$, at 1269 nm for 2a-3c and the absence for 1b in oxygen- and air-saturated dichloromethane (and for 2a-3c under argon) is further evidence for the triplet nature in these cases. The quantum yield Φ_{Δ} , which is a minimum for that of intersystem crossing (Φ_{isc}), is substantial, $\Phi_{\Delta} = 0.3$ –0.5 (Table 2). The enhancement of Φ_{Λ} on going from air- to oxygen-saturated dichloromethane is due to the short $\tau_{\rm T}$ of 0.4–1.2 μ s which, under air, leads to more deactivation without quenching. It is worth mentioning that for the photoconversion of 1-nitro-2naphthaldehyde into the nitrosonaphthoic acid it has been reported that UV-Vis spectroscopy does not reveal any observable *aci*-nitro form but a triplet state with τ_{T} in the ns range (33).

The aci-nitro form was also detected as major long-lived transient. For 4d in aqueous solution at pH 3-4 (absence of a buffer) or at pH 7 (phosphate buffer) the changes in ΔA_{aci} are small (data not shown). The aci-nitro form of 5b (Fig. 6a) can be considered as a clean example with only one component, while in DMSO-water a conversion into another aci-nitro form is indicated (Fig. 6b). 6a is more complex in that the aci-nitro form is converted into another in which λ_{aci} is redshifted (Fig. 7). Moreover, the presence of water has a large effect, *i.e.* a much shorter τ_{aci} value. This effect is new and points to a water-mediated behavior in contrast to that of most other cases, e.g. for **1a-c** or **5a/b**. Plots of ΔA_{420} vs log time are shown in Fig. 8 for 6a in acetonitrile, DMSO and 1:1 mixtures with H₂O. The ΔA_{aci} values as a measure of $\varepsilon_{410} \times \Phi_{aci}$ of 1–3 or 4a-e in acetonitrile are 0.05-0.1 and 0.3 (Table 3), respectively.

The results so far refer to room temperature and almost nothing is known concerning the effect of temperature on the kinetics of the 2-nitrobenzyl function. The τ_{aci} values of **1a–6b** become shorter at elevated temperatures and semilogarithmic plots of $1/\tau_{aci}$ vs 1/T are linear (Fig. 9). In those cases, where a second shorter lived τ_{aci} component was detected at room temperature, its contribution became smaller or not observable



Figure 7. Transient absorption spectra of **6a** in (a) DMSO and (b) DMSO-H₂O (1:1, pH 4) at 20 ns (\bigcirc), 1 μ s (\triangle), 10 μ s (\square), 1 ms (\blacktriangle) and 100 ms (\blacklozenge) after the 308 nm pulse.



Figure 8. Plots of ΔA_{460} vs log time for **6a** in acetonitrile (a: circles), DMSO (triangles), dry (b: open) and 1:1 mixtures with H₂O (c: full).

Table 3. Molar absorption coefficient $(10^3 \times m^{-1} \text{ cm}^{-1})$, relative efficiency and quantum yield of conversion.*

Comp.	\$254/\$308	$\Phi_{ m d}\dagger$	$\Delta A_{ m aci}$ ‡
1a	7.8/2.0	0.1	0.1
1b	10.3/2.5	0.12 (0.15)§	0.1
1c	15.2/3.1	0.12	0.1
2a	10.7/6.3	0.08	< 0.08
2b	11.0/5.7	0.1	< 0.08
2c	18.1/6.7	0.1	< 0.08
3a	11.0/4.5	0.1	< 0.08
3b	13.5/4.7	0.1	< 0.08
3c	19.3/5.1	0.08	< 0.08
4a	14.4/6.6	0.23	0.3
4b	16.2/7.1		0.3
4c	20.7/8.7	0.2	0.3
4d	15.0/5.7	0.2	0.3
4e	15.3/5.1	0.2	0.2
5a	15.8/5.4	0.2	0.2
5b	16.1/5.9	0.2	0.2
6a	21.1/5.2		0.2
6b	33.3/6.6		0.11
7	13.4/4.2	< 0.02	< 0.02

*In air-saturated acetonitrile, except where indicated. †Using $\lambda_{irr} = 254$ nm, see Fig. 10. ‡Relative value at 410 nm and 10 μ s after the pulse. §In DMSO-water.



Figure 9. Plots of log $1/\tau_{aci}$ vs 1/T for 1b (circles), 4b (triangles), 5b (squares) and 6a (diamonds) in DMSO (open) and ethanol (full).

(in yield) at elevated temperatures. The activation energy (E_a) and the A-factor (A_0) of the Arrhenius dependences for the reaction from HA to HB (Scheme 1) are listed in Table 4. The E_a value for 2-nitrobenzyl acetate 1a in solution can be compared with a calculation of $E_a = 62 \text{ kJ mol}^{-1}$ in the gas phase (13). Generally, E_a and A_0 do not vary much with the nature of the solvent. The obvious effects are a markedly larger A_0 value for 5 and smaller E_a when water is mixed with DMSO. Therefore, one can assume a transition state with an

Table 4. Activation energy (in kJ mol⁻¹) and preexponential factor (in s⁻¹).*

Comp.	MeCN: <i>E</i> _a	$\log A_0$	DMSO: <i>E</i> _a	$\log A_0$	EtOH: <i>E</i> _a	$\log A_0$
1a	48	11.1	50	10.9	50	10.3
1b	52	10.8	51	10.6	50	10.3
1c	50	10.9	49	10.6	50	10.4
3b			51	10.7		
4a	50	10	50	9.6	51	9.2
4b	50	10	50	9.4	52	9.4
4c	48	9.8	50	9.4	51	9.2
4d					52	10.3
5a/b	50	13	50	12.9	50	13.2
6a			48 (44)†	9.0	50	11.3
6b			52 (46)	8.5	51	10.7

*From plots of log $1/\tau_{aci} vs 1/T$, cf. Fig. 9. †Values in parentheses refer to DMSO-water (1:1).

increased dipole compared to the *aci*-nitro-intermediate. It is noteworthy that values in a similar range, $A_0 = 10^{11}-10^{13} \text{ s}^{-1}$ and $E_a = 48-54 \text{ kJ mol}^{-1}$, were found for 2-nitrobenzyl alcohol and two derivatives in the same solvents (26). For 1-(2-nitrophenyl)ethyl ether at pH 8.5 a much smaller value of $E_a = 25 \text{ kJ mol}^{-1}$ has been reported (17).

Continuous irradiation

UV irradiation in acetonitrile (air- or argon-saturated at room temperature) leads to substrate decomposition, as registered by HPLC for the cases of 1a-4e. The spectra of nitrobenzyl compounds 1a-c are characterized by two characteristic wavelengths (quasi-isosbestic points) at 240 and 290 nm, absorption decreases between and increases in the range above 300 nm. For 4,5-dimethoxynitrobenzyl 2 and 4,5-methylenedioxynitrobenzyl compounds 3 the quasi-isosbestic points are shifted to 320 and 370 nm (27). The spectral changes in 2a-4e are, in principle, similar and redshifted with respect to methoxy nonsubstituted 1.

The quantum yield of decomposition (Φ_d) was obtained either from the increase in absorbance at an appropriate wavelength, *e.g.* 260 nm (Fig. 10a), or the decrease in the



Figure 10. Change in (a) A_{obs} at 400 nm and (b) substrate concentration *vs* time due to 254 nm photoconversion of **1a** (Δ), **1b** (\Box), **1c** (\diamond), **4a** (\bigcirc , \bullet), **4b** (\blacktriangle) and **4c** (\blacksquare) in acetonitrile.

concentration using HPLC data (Fig. 10b). The literature values are $\Phi_d = 0.08$ for **1b** in acetonitrile and $\Phi_d = 0.15$ for 1a in buffered DMSO-water (6,12). For comparison, $\Phi_{\rm d} = 0.1$ for **2b** in acetonitrile using $\lambda_{\rm irr} = 313$ nm. $\Phi_{\rm d}$ is typically 0.2 for 4/5 type acids (Table 3) and zero only for 7, which contains the rigid amino group and shows no transients at all. The photodecomposition of 1a yields 2-nitrosobenzaldehyde and acetic acid, that of 1b or 1c benzoic acid or 4-methylbenzoic acid, respectively. These three organic acids are present in neutral aqueous solution as anion due to pK_a ca 3-5. They are also formed upon irradiation of 2/3, together with the 4,5-dimethoxy and 4,5methylenedioxy derivatives of 2-nitrosobenzaldehyde. It has been reported that the quantum yield of photocleavage is markedly lower than the unsubstituted 2-nitrobenzyl caged compound (23).

Nitroso compounds, which are formed upon irradiation of nitrobenzyl compounds may yield the corresponding dimer, e.g. at low temperatures (1,34), but the equilibrium between the monomer and the corresponding dimer is difficult to predict. Detection of a dimer within ca 10 s has been reported for a 2-nitrophenylethoxyethyl phosphate (17), but the substrate concentrations under these IR measurements in water are typically a thousand times larger than for UV-Vis spectroscopy. It has been argued that the absorption at 320 nm of 2-nitrosobenzaldehyde is due to the dimer and at 750 nm due to the monomer (4,13,17), which however also absorbs around 300 nm (6). Arguments against thermal dimer formation under the spectroscopic conditions of nitrosobenzenes have been given (4,19-21). Here, no dimers could be detected by UV-Vis spectroscopy and HPLC for any photoproduct in acetonitrile under the applied conditions. The photoconversion into 2-nitrosobenzaldehyde and benzoic acid is the main process, e.g. for 1a-4e; other peaks were only registered upon prolonged irradiation, e.g. for 5a-6b.

General mechanism

The photoreaction of compounds with 2-nitrobenzyl entity is initiated by intramolecular benzylic H-atom transfer to the nitro group, reactions 1-3 in Scheme 1. Nitroarenes generally show a substantial Φ_{isc} and intramolecular H-atom transfer takes place in the triplet state. In specific cases the H-atom can also be transferred in the excited singlet state (1,21). Hydrogen shift gives the aci-nitro form as main observable intermediate. For 2-nitrobenzyl aryl esters (6) and 2-nitrobenzyl-methyl-ether (20) in dry acetonitrile it has been argued that no deprotonation takes place. More than one aci-nitro form has been proposed in many cases (9-15), but in some cases the decay at λ_{aci} is mono-exponential (13). The known reason for an increase in the 430-500 nm absorbance (growin component) in the μ s range is the equilibrium between HA and A⁻ in aqueous solution or mixtures of organic solvents with water; formation of A⁻ is delayed and its maximum can be redshifted (6,20,22). The aci-nitro form can be present in any of the four isomers, two concerning the orientation of the aci-nitro group (tautomeric forms) and two concerning the orientation of the benzylic/quinoid group (diastereomers). Only one or two decay components are usually observed (12,13). The two stereoisomers (HA_E and HA_Z) can be distinguished, whereby it was proposed that the longer lived component is stabilized by the interaction of the α -hydrogen with an oxygen of the HA form (6). Earlier, we have considered sterical reasons as an alternative explanation (12). The further rearrangement leads via the cyclic benzisoxazoline (HB in Scheme 1) and the hemiacylal (HC) into the 2-nitroso product OCRArNO and HX (MeCO₂H, PhCO₂H and p-TolCO₂H for acetic, benzoic and toluic acid esters, respectively). For the specific case of 2-nitrobenzyl methyl ether in aqueous solution these species have recently been detected (20). In aqueous solution the equilibria (HB/B⁻ and HC/C^{-}) could be important and eventually the leaving group HX is released. The most frequently concluded mechanism is that the aci-nitro acid form (HA) is the precursor of both OCRArNO and HX. Walbert et al. suggested that the anion of the 2-(2-nitrophenyl)ethyl acetate-caged compounds undergoes β -elimination and the acid nitrosobenzene formation (22).

It is a widely accepted assumption among researchers using caged compounds, that the decay of the aci-nitro intermediate HA (Scheme 1) is the rate-limiting step in the photorelease of the active species (35,36). However, detailed studies on model systems (20) have revealed that this assumption is not necessarily correct. If the leaving group is methoxy, the release is rather slow (about τ_{aci} = 1–100 s) and strongly dependent on the reaction condition. The rate changes more than 2 orders of magnitude as a function of pH. Earlier studies on a caged glutamate (37) reveal that the release of glutamate from α -carboxynitrobenzyl glutamate in phosphate buffer at pH 7 is as fast as the decay of the *aci*-nitro intermediate and τ_{aci} is *ca* 20 μ s. From these data one can only get an upper limit for the lifetime of the hemiacylal HC, which directly releases the free acid. The difference in behavior between compounds with methoxy or carboxy as leaving groups is in good agreement with the fact that carboxylates are much better leaving groups than alcoholates, or that carboxylic acids are much better leaving groups than alcohols. We observed lifetimes of 0.001-160 ms for the aci-nitro intermediates of different compounds. The assumption that the decay of the aci-nitro intermediate is the rate-limiting step seems to be likely in many cases but is not true in all.

Properties of 2-nitrobenzyl acetate, benzoate and toluate

For 2-nitrobenzyl benzoate 1b two isomers can be distinguished kinetically (6). Two components of the aci-nitro forms have also been proposed in other cases (13,14). It has been argued that the longer lived component of 1b is stabilized by the interaction of the α -hydrogen with an oxygen of the AH form (6). For 2-nitrophenylethyl acetates the redshift of the absorption maximum and the longwavelength side of aci-nitro has been attributed to the conversion of AH into A⁻ (22). We suggest that both reasons, depending on the structure and the medium, account for the two components: hydrogen bonding, as in dichloromethane (Fig. 1b), dry DMSO or dry acetonitrile, and deprotonation. This latter reaction (2 in Scheme 1) is proposed to account for the two aci-nitro species of 1a-c in mixtures of DMSO (Fig. 2a,b), acetonitrile or ethanol with water (Table 1). Apparently, the redshift is larger for 10:1 mixture than in neat water.

Properties of 4,5-dimethoxynitrobenzyl and 4,5-methylenedioxynitrobenzyl compounds

The 4,5-methylenedioxy or methoxy groups lead to several surprising effects of the transients. One is the broad spectrum (Fig. 3a,b), one the much shorter lifetime and another the effect of quenching by oxygen and conversion of triplet into $O_2(^1\Delta_{\alpha})$. Φ_{Λ} of 2/3 in dichloromethane (Table 2) account for 30-50% of the absorbed photons. These results are not in agreement with the efficiency of aci-nitro formation. The suggested explanation is that the transient of 2/3 is assigned to a triplet state, as illustrated in Scheme 2. The photoconversion of 2-nitrobenzaldehyde into 2-nitrosobenzoic acid occurs via the aci-nitro form but does not reveal any triplet state in the ns time scale (1). For 1-nitro-2-naphthaldehyde, however, Dvornikov et al. showed that the photoconversion into the nitroso acid does not reveal any aci-nitro form but the precursor; this triplet state has a lifetime of 50-600 ns at room temperature and 65 ms in ether/pentane/ethanol at -196°C (33). The relatively high $\Delta A_{\rm T}$ values of the triplet in 2/3 and the lower $\Delta A_{\rm aci}$ values of the longer lived transient(s) are compatible with comparable Φ values and much higher ε values for T-T absorption than for absorption of the aci-nitro form. The charge transfer, induced by the electron donating groups in positions 4 and 5 shifts not only the absorption maximum to ca 350 nm but also lowers the triplet level. For 2/3 we propose two reaction sequences with comparable yield: one is as for 1 and the other is charge-transfer induced, but chemically inert. It is worth mentioning that also for 4,5-dimethoxy- and 4,5-methylenedioxy-2-nitrobenzyl alcohol, in contrast to parent 2-nitrobenzyl alcohol, a triplet state with similar features (albeit low Φ_{Λ}) has been detected (26). For 4-amino-4'-nitroarenes, a related donor-substituted aromatic nitrocompound, a charge transfer triplet state with a lifetime of a few ns and a molar absorption coefficient in the range of $\varepsilon_{\rm T} = (2-4) \times$ $10^4 \text{ m}^{-1} \text{ cm}^{-1}$ at 600–900 nm are known (38).

Properties of nitro and dinitro compounds: 4-6

The results for α -carboxy-4,5-methylenedioxynitrobenzyl compounds **4** are also in accord with Scheme 2. The short-lived transient of **4b** (Fig. 4a,b) is assigned to the triplet state due to the effect of oxygen. The decay at λ_{aci} is mono-exponential, introduction of a carboxy group in **4** gives rise to rather long τ_{aci} values which are similar for **a**-**c**, *i.e.* a change in the leaving group is unimportant. These compounds show the largest spectral changes among the 2-nitrobenzyl esters (27). We therefore tried to detect the formation of OCRArNO (R: CO_2H) of **4b**. In fact, after complete transient decay around 400 nm, a conversion into a permanent absorption was observed (Fig. 4a,b). T-T absorption and *aci*-nitro form absorption are also overlapping for **4d** (Fig. 5a,b) and **4e**, containing amino acid building blocks. A dimethyl compound similar to **4d** was used in the photoreduction of cytochrome *C* (7). Some effects of substitution were reported (13).

Bis-nitrophenyl acetates **5b** (Fig. 6a,b) and **5a** reveal monoexponential decay at λ_{aci} which is slightly redshifted with respect to a classical *aci*-nitro spectrum. A rather short lifetime (Table 1) can be attributed to the sterical stress due to the bulky groups. Introduction of a carboxy group gives rise to a complex behavior for **6a**, where a grow-in within *ca* 1 μ s and a redshift of λ_{aci} was detected (Fig. 8a,b). This conversion of an initial *aci*-nitro form into a second with a rather long τ_{aci} is probably due to the two carboxy groups as otherwise the structure of **6a** is identical to **5a**. The reason cannot be the carboxy group as such, as this conversion does not occur for **4a**–e. One possibility could be proton transfer between the *aci*nitro and meta-carboxy groups. The reason is not fully understood.

In principle, the triplet state could be the precursor of the *aci*-nitro form or not. If the triplet state is part of the pathway into the nitroso product, then quenching by oxygen should lower Φ_d . The triplet state seems not to be in the pathway *via* the *aci*-nitro form as Φ_d is not affected by the presence or absence of oxygen. This is different to the case of 1-nitro-2-naphthaldehyde, where involvement of the triplet state, in addition to another (singlet) pathway to the *aci*-nitro form, had been concluded, but only 30% of the excited 1-nitro-2-naphthaldehyde molecules are deactivated *via* the observed triplet state (33).

CONCLUSION

The photoreactions of 2-nitrobenzyl-type into nitroso compounds and release of the leaving groups acetic acid (a: 1, 2, 3 and 4), benzoic acid (b) and methyl-benzoic acid (c) were studied. The role of the organic acid HX within a group is minor, Φ_d is relatively small and no principal changes were found for τ_{aci} under the same excitation conditions. Surprisingly, the methylenedioxy and methoxy groups lead to



Scheme 2.

population of a triplet state. In most of the other cases both triplet state and aci-nitro form are observable in the ns/µs and μ s/ms range, respectively. Group 1 and 5 compounds follow the classical pathway (reactions 1-6 in Scheme 1), where only *aci*-nitro isomers were detected. For group 2/3the triplet state is a major transient. The aci-nitro form, but not the triplet state, is postulated to be an intermediate on the pathway in the nitroso product. For a-carboxy-4,5methylenedioxynitrobenzyl compounds 4 both the triplet state and the aci-nitro form were observed, but only the latter is intermediate on the pathway into the nitroso product. The effects of temperature on the decay kinetics of the aci-nitro form reveal Arrhenius dependences and common trends for a given molecule in solution. The changes in τ_{aci} at room temperature are due to both, E_a and A_0 . E_a is around 50 kJ mol⁻¹ for several compounds in DMSO, acetonitrile or ethanol, but A_0 varies with medium and structure; it is smallest for 6 and largest for 5. The solvent plays a decisive role in the properties of the various steps, especially the presence of water. When deprotonation can be excluded in dichloromethane, intrinsic reasons have to be assumed. On the other hand, in neutral aqueous solution the aci-nitro form is present as anion.

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