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Photogeneration of quinone methide-type intermediates from pyridoxine and derivatives

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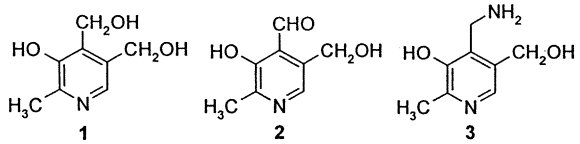
Abstract

The photochemical generation of novel quinone methide-type intermediates has been observed upon photolysis of Vitamin B_6 (pyridoxine, **1**) and its derivatives **7** and **8**. Irradiation of **1** or **7** in 1:1 CH_3OH/H_2O gives the corresponding methyl ethers ($\Phi_p = 0.18$ and 0.21, respectively), consistent with nucleophilic attack of methanol on *o*-quinone methide-type intermediates. Similarly, photolysis in aqueous CH_3CN with ethyl vinyl ether resulted in the regioselective formation of the respective chroman products through [4 + 2] cycloaddition. Although laser flash photolysis (LFP) studies point to formation of two different *o*-quinone methide-type intermediates upon irradiation of either **7** or **8** in neutral aqueous solution, only one such reactive species is observed in alkaline solutions. These intermediates necessarily arise from formal loss of water. The mechanism by which this occurs is dependent upon the pH of the solution. In alkaline solution formation of the quinone methide-type intermediate occurs by dehydroxylation of the excited state phenolate. In neutral solution the reactive species may be formed either by ESPT or by excited state intramolecular proton transfer (ESIPT) between the phenol and the benzylic alcohol, followed by loss of water.

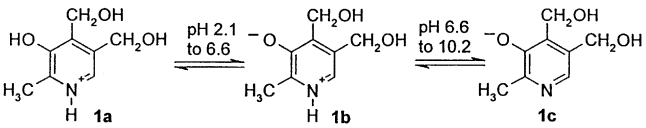
Keywords: *o*-Quinone methide; Pyridoxine; Vitamin B_6; Excited state proton transfer

1. Introduction

Vitamin B_6 (pyridoxine, **1**) plays an important role in biological systems [1]. A deficiency of **1** in the body can lead to a skin disorder (dermatitis acrodynia) [2], while large doses can result in seizures, ataxia, and other neurotoxic effects [3,4]. Pyridoxine (**1**) is the dietary precursor for pyridoxal (**2**), an important coenzyme involved in amino acid metabolism, and a cofactor in the phosphorylation of glycogen. Similarly, **2** and pyridoxamine (**3**) are fundamental in transamination reactions required for the oxidative degradation of amino acids.



Due to their biological importance, the basic photophysical properties of **1**–**3** have been extensively studied. Through UV–VIS studies Harris et al. [5] have shown the existence of three different forms of **1**, depending upon pH (Eq. (1)) and predicted that the zwitterion (**1b**) predominates at pH 7, rather than the neutral form. Similarly, the fluorescence spectra of **1**–**3** and other analogues have been thoroughly analyzed [6,7] and the fluorescence quantum yields from **1** and **3** have been determined [8]. Interestingly, while the photophysics are reasonably well understood, the photochemistry arising from excitation of **1** and its derivatives is limited to a few examples. These include the generation of an oxirane intermediate [6], rearrangements through benzvalene-type intermediates and formation of self-adducts [9], and oxidative photodegradation [10,11].



(1)

Our interest in the photochemistry of these systems arises from our work on the generation of quinone methides upon photolysis of *o*-, *m*- and *p*-hydroxybenzyl alcohols and hydroxybenzhydrols (e.g. formation of *o*-quinone methide (**4**) from *o*-hydroxybenzyl alcohol, Eq. (2)) [12–19]. These studies have shown that the presence of the hydroxybenzyl

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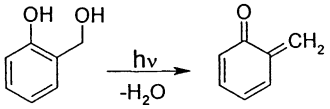
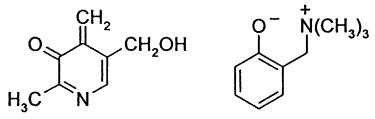
alcohol-type moiety allows for the efficient formation of the reactive intermediates through a formal loss of water upon excitation. Given the presence of these moieties on **1**, we believed that a similar *o*-quinone methide-type intermediate (**5**) could be generated upon its irradiation. Also, as **1** contains two benzylic alcohols (one *ortho* and one *meta* to the phenol), this study also provided an opportunity to investigate the relative contributions of *o*- and *m*-quinone methide formation to the overall photochemistry. Indeed, previous studies have shown that the quantum yields of *o*- and *m*-quinone methide formation from *o*- and *m*-hydroxybenzhydrol, respectively, are almost equivalent ($\Phi_p = 0.46$ [15] and 0.40 [20], respectively).



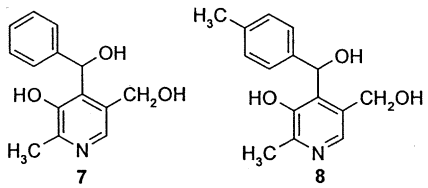
4 (2)

Interestingly, literature precedent exists for the thermal formation of **5** from **1** via high temperature dehydration in several nucleophilic solvents [3]. It was predicted that an enzyme mediated protonation of the benzylic alcohol *ortho* to the phenolate (**1b** at physiological pH) could result in the formation of **5** in vivo, which, due to its highly reactive nature, could be responsible for nerve damage and other toxic reactions observed upon intake of large doses of **1** [3,4]. These results provided further incentive to attempt to photogenerate a quinone methide intermediate from **1**. Similar thermal formation of a quinone methide (**4**) has been reported [21] from **6** at 35 °C, where the presence of the phenolate is believed to promote quinone methide formation due to its electron donating ability.



5 **6**

The quinone methide from **1** is expected to be difficult to detect due to its anticipated low extinction coefficient at wavelengths above 300 nm. For this reason, laser flash photolysis (LFP) experiments were conducted on derivatives **7** and **8**, as the added α-phenyl groups will extend the conjugation of the system and allow for ready observation of the suspected quinone methides if they are generated. A preliminary report of the present work has previously been disseminated [22].



7 **8**

2. Experimental details

2.1. General

^{1}H NMR spectra were recorded on either a Bruker AC 300 (300 MHz) or a Bruker AM 360 (360 MHz) instrument using D_2O, $CDCl_3$ or DMSO-d_6 as solvents. Mass spectra were determined on either a Finnegan 3300 (CI) or a Kratos Concept 1H (FAB and HRMS). Melting points were determined on a Reichart 7905 melting point apparatus (uncorrected). UV–VIS spectra were recorded using a Varian Cary 1 or 5 spectrophotometer. Preparative thin layer chromatography (Prep TLC) was performed utilizing 1000 μm silica gel plates from Analtech and the solvent systems listed in each experiment. pH measurements were taken using a Corning 140 pH meter. THF was dried over Na and distilled prior to use.

2.2. Materials

Grignard reagents were prepared by adding either benzyl bromide or 4-methylbenzyl bromide to 1.5 eq. of clean, dry Mg turnings stirring in dry THF under N_2 at 0 °C and allowing the solution to warm to room temperature. Pyridoxine (**1**) and pyridoxal (**2**) were purchased from Aldrich as the HCl salts and were used as received.

2.2.1. α-Phenylpyridoxine (7)

A solution of phenyl magnesium bromide (∼170 mmol in THF—prepared as above) was added dropwise to a clean, dry, cooled (0 °C) three-neck flask charged with 4 g (19.6 mmol) of pyridoxal (**2**) in 150 ml of dry THF and purged with N_2. The solution was then allowed to stir overnight under gentle reflux. After this time, the reaction was quenched by the addition of NH_4Cl and the solution brought to pH 7. The reaction mixture was extracted with CH_2Cl_2 and then washed with aqueous HCl (0.1N) to isolate the product as the HCl salt in the aqueous phase. Following removal of water under reduced pressure, the HCl salt of **7** was obtained. This was dissolved in ethanol and filtered to remove any residual inorganic salts. After removal of ethanol, the product was dissolved in a minimum of hot methanol and diethyl ether was added to precipitate the desired product as a gray-brown powder (2.6 g, 47%); mp 173–176 °C; ^{1}H NMR (HCl salt) (300 MHz, D_2O) $\delta = 2.51$ (s, 3H, CH_3 on pyridine ring), 4.37 (d, 1H, $J = 14.7$ Hz, methylene H from CH_2OH), 4.59 (d, 1H, $J = 14.7$ Hz, methylene H from CH_2OH), 6.27 (s, 1H, CH), 7.27 (m, 5H, ArH), 8.00 (s, 1H, pyridine Ar H); MS: (FAB) *m*/*z* 246 (M–Cl). HRMS Calcd. for $C_{14}H_{16}NO_3^+$ 246.1130; found 246.1143. Anal. Calcd.: C (59.68%), H (5.72%), N (4.97%); found C (58.36%), H (5.67%), N (4.70%).

2.2.2. α-(4-Methylphenyl)pyridoxine (8)

A solution of 4-methylphenyl magnesium bromide (∼78 mmol in THF—prepared as above) was added dropwise to

a clean, dry, cooled (ice-bath) three-neck flask charged with 2 g (9.8 mmol) of pyridoxal (**2**) in 150 ml of dry THF and purged with N_2. The solution was allowed to stir overnight under gentle reflux. Work-up (carried out as for **7** above) yielded a brown powder (1.25 g, 43%), which was shown by ^{1}H NMR to be the desired product **8**; mp 179–181 °C; ^{1}H NMR (HCl salt) (300 MHz, D_2O) $\delta = 2.17$ (s, 3H, CH_3 on phenyl), 2.50 (s, 3H, CH_3 on pyridine ring), 4.33 (d, 1H, $J = 14.7$ Hz, methylene H from CH_2OH), 4.58 (d, 1H, $J = 14.7$ Hz, methylene H from CH_2OH), 6.21 (s, 1H, CH), 7.12 (m, 4H, ArH), 7.99 (s, 1H, pyridine Ar H); MS: (FAB) *m/z* 260 (M–Cl). HRMS Calcd. for $C_{15}H_{18}NO_3^+$ 260.1287; found 260.1294.

2.3. Product studies

All preparative photolyses were carried out in a Rayonet RPR 100 photochemical reactor equipped with 254 nm lamps. The solutions were contained in quartz tubes (∼200 ml) which were cooled to ≤15 °C with tap water by means of an internal cold finger. All solutions were purged with either argon or oxygen (via a stainless steel needle) for 5 min prior to irradiation and for the entire time of the irradiation (5–15 min) to promote stirring and maintain the atmosphere. General work-up following photolysis involved extraction of the solvent system (typically CH_3CN/H_2O or CH_3OH/H_2O) with CH_2Cl_2, followed by drying of the organic layer and evaporation of the solvent under reduced pressure. All pH studies list the pH of the aqueous phase prior to mixing with CH_3CN or CH_3OH. In these studies, the solution was brought to ∼pH 7 following irradiation. The irradiation products were separated by preparative TLC and analyzed by MS and ^{1}H NMR. In all cases, control experiments (≤15 °C, in the dark) were conducted to determine the contribution of thermal processes to the observed reactivity.

2.3.1. Photolysis of **1** in 1:1 CH_3OH/H_2O

A solution of 54 mg of **1** in 80 ml of 1:1 CH_3OH/H_2O was photolyzed (eight lamps) for times up to 15 min. Solutions which were not irradiated showed no conversion to the methyl ether (no thermal reaction). ^{1}H NMR analysis showed the methyl ether **9** as the only product at these irradiation times. Preparative TLC separation (100% ethyl acetate) was used to obtain a pure sample of the methyl ether which has previously been identified [23,24]; ^{1}H NMR (free base) (300 MHz, DMSO-d_6) $\delta = 2.34$ (s, 3H, CH_3 on phenyl), 3.25 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 7.93 (s, 1H, ArH). HRMS Calcd. for $C_9H_{13}NO_3$ 183.0895; found 183.0893.

Solutions of 54 mg **1** in 80 ml 1:1 CH_3OH/H_2O at several pH values (1.0, 2.2, 3.5, 5.2, 6.7, 7.5, 9.1, 10.3, 11.2 and 12.0) were photolyzed at 254 nm for times up to 15 min. The product obtained in each case was shown via ^{1}H NMR to be identical to methyl ether (**9**).

2.3.2. Photolysis of **1** in 1:1 CH_3CN/H_2O with added ethyl vinyl ether (EVE)

A solution of 80 mg **1** dissolved in 80 ml of 1:1 CH_3CN/H_2O was photolyzed with EVE (1 ml, 10 mmol) at 254 nm (eight lamps) for 5 and 15 min. Following work-up, the resultant product mixture was found to contain both residual **1** and chroman-like **12** as identified by ^{1}H NMR. Preparative TLC (100% ethyl acetate) was used to obtain a pure sample of **12**; ^{1}H NMR (free base) (300 MHz, $CDCl_3$) $\delta = 1.05$ (t, 3H, $J = 7.4$ Hz, CH_3 of ethoxy group), 1.95 (m, 2H, CH_2 next to ethoxy group), 2.25 ppm (s, 3H, CH_3 on phenyl ring), 2.70 ppm (t, 2H, $J = 7.4$ Hz, CH_2 *para* to N), 3.71 ppm (qq, 2H, $J = 7.4$, 9.6 Hz, CH_2 of ethoxy group), 4.48 (s, 2H, CH_2 on phenyl ring), 5.33 (t, 1H, $J = 3.7$ Hz, H geminal to ethoxy group), 7.78 (s, 1H, ArH). HRMS Calcd. for $C_{12}H_{17}NO_3$ 223.1208; found 223.1213.

Solutions of 80 mg **1** in 80 ml of 1:1 CH_3CN/H_2O at several pH values (1.0, 1.7, 3.5, 5.2, 6.5, 7.0, 9.2, 10.2 and 12.0) were photolyzed with 1 ml of EVE (1 ml, 10 mmol) at 254 nm (eight lamps) for 5 and 15 min. Irradiation in solutions of pH ≃ 1 and >7 showed none of the chroman product due to decomposition of the EVE, while irradiation at pH between 3.5 and 7 showed ∼8% product formation.

2.3.3. Photolysis of **7** in 1:1 CH_3OH/H_2O

A solution of 54 mg of **7** in 80 ml of 1:1 CH_3OH/H_2O was photolyzed at 254 nm (eight lamps) for times up to 15 min. ^{1}H NMR analysis showed methyl ether (**11**) as the only product at these irradiation times, although photolysis for greater than 15 min showed the appearance of secondary photoproducts (not identified). Preparative TLC separation (100% ethyl acetate) was used to obtain a pure sample of the methyl ether; ^{1}H NMR (free base) (300 MHz, $CDCl_3$) $\delta = 2.48$ (s, 3H, CH_3 on phenyl), 3.48 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 5.92 (s, 1H, CH), 7.32 (s, 5H, phenyl H), 7.87 (s, 1H, ArH), 8.72 (s, 1H, phenol); mass spectrum (CI) *m/z* 260 ($M^+ + 1$). HRMS Calcd. for $C_{15}H_{17}NO_3$ 259.1208; found 259.1213.

2.3.4. Photolysis of **7** in 1:1 CH_3OH/H_2O with added ethyl vinyl ether (EVE)

A solution of 50 mg **7** dissolved in 80 ml of 1:1 CH_3CN/H_2O was photolyzed with 1 ml (10 mmol) of EVE at 254 nm (eight lamps) for 15 min. Following work-up, the resultant product mixture was found to contain diastereomers **13** and **14**, as well as residual **7**. Preparative TLC (100% ethyl acetate) was used to obtain a pure sample of the chroman-like diastereomers **13** and **14**; ^{1}H NMR (free base) (360 MHz, $CDCl_3$) $\delta = 1.07$ (t, 3H, $J = 7.1$ Hz, CH_3 of ethoxy group, diastereomer **1**), 1.20 (t, 3H, $J = 7.1$ Hz, CH_3 of ethoxy group, diastereomer **2**), 2.11–2.48 (m, 2H, CH_2 next to ethoxy group, diastereomers **1** and **2**), 2.49 (s, 3H, CH_3 on phenyl ring, diastereomer **2**), 2.50 (s, 3H, CH_3 on phenyl ring, diastereomer **1**), 3.46–3.95 (m, 2H, CH_2 of ethoxy group, diastereomers **1** and **2**), 4.15 (d, 2H, $J = 4.1$ Hz, CH_2 on phenyl ring, diastereomer **1**), 4.18 (s,

2H, CH_2 on phenyl ring, diastereomer **2**), 4.38 (dd, 1H, $J = 5.3$, 7.9 Hz, CH with phenyl, diastereomer **1**), 4.44 (t, 1H, $J = 6.5$ Hz, CH with phenyl, diastereomer **2**), 5.13 (dd, 1H, $J = 2.4$, 6.6 Hz, diastereomer **2**), 5.23 (dd, 1H, $J = 2.5$, 4.8 Hz, diastereomer **1**), 7.05–7.09 (m, 5H, phenyl ArH, diastereomer **1**), 7.15–7.31 (m, 5 H, phenyl ArH, diastereomer **2**), 8.02 (s, ArH *ortho* to N, diastereomer **2**), 8.03 (s, ArH *ortho* to N, diastereomer **1**); mass spectrum (CI) *m/z* 300 ($M^+ + 1$). HRMS Calcd. for $C_{18}H_{21}NO_3$ 299.1521; found 299.1524.

2.4. Quantum yields

Product quantum yields for the photosolvolysis (Φ_p) of **1** was determined by comparison of the ^{1}H NMR yields at low conversions to a reference reaction, the analogous photomethanolysis of an equimolar amount of *o*-hydroxybenzhydrol in 1:1 CH_3OH/H_2O ($\Phi_p = 0.46$ [15]). The Φ_p for **7** was determined by comparison of the ^{1}H NMR yields at low conversion to the photomethanolysis of **1**. All values are the result of at least three independent irradiations.

2.5. LFP

All transient spectra and kinetic measurements were recorded using nanosecond LFP with excitation by either a Spectra Physics YAG laser (Model GCR-12, 266 nm excitation) or a Lumonics excimer laser (Model EX-510, 308 nm excitation). Samples of OD $\approx$ 0.3 at the excitation wavelength were prepared and irradiated in quartz cells. Flow cells were used for spectra in order to eliminate complications from long-lived intermediates, while static cells were used for the quenching studies. Flow cell solutions were purged continuously with either O_2 or N_2, while static cells were purged for a minimum of 10 min prior to irradiation. Due to the long transient lifetimes observed in both types of experiments, it was not always possible to obtain a trace that returned to the baseline.

3. Results and discussion

3.1. UV–VIS studies

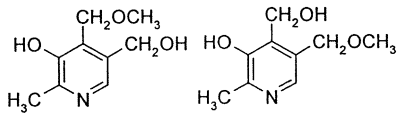
UV–VIS spectrophotometry was used to compare **7** and **8** to the parent compound **1**, in order to determine if the addition of the α-phenyl group had any effect on the absorption characteristics of the pyridoxine chromophore. The absorption spectra of **1** were the same as those recorded by Harris et al. [5] in aqueous solution at pH 1, 7 (0.01 M buffer) and 12. The absorption spectra displayed by **7** and **8** were identical at all three pH values and showed only a slight red shift (~2 nm) in λ_{max} at pH 1 and small blue shifts (~2 nm) at pH 7 and 12 compared to **1**. This indicates that the added phenyl rings have little effect on the absorption spectrum on the chromophore of **1**. Thus, LFP studies conducted on **7** and **8** can be expected to directly reflect the excited state chemistry of **1**.

3.2. Product studies

3.2.1. Aqueous methanol

Photolysis of pyridoxine (**1**) in 1:1 H_2O–CH_3OH (254 nm, eight lamps, 5 min) gave methyl ether (**9**) as the only product in ~8% yield (assigned by NOE experiments vide infra). No product formation was observed in solutions which were not irradiated, thereby ruling out reaction through thermal pathways. Irradiation for longer periods of time resulted in higher yields with no secondary photoproducts being observed. Product yields were unaffected on irradiation under O_2 or N_2. This is consistent with reaction via the singlet, as already demonstrated for related reactions [15,16].

Exhaustive photolysis of **1** under the same conditions as above, followed by Prep TLC, allowed for the isolation of methyl ether (**9**) and its characterization by ^{1}H NMR. No evidence was found for **10**, although previous work by our group [15,16] has shown formation of the respective methyl ethers from irradiation of the closely related *m*-hydroxybenzyl alcohol and *m*-hydroxybenzhydrol under the same conditions.



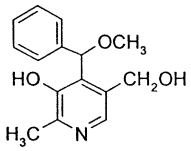
9 **10**

As evidenced by the appearance of a singlet at δ 3.48 ppm in the ^{1}H NMR spectrum, irradiation of **7** in 1:1 CH_3OH/H_2O also yielded a single methyl ether (**11**). Similar to **1**, longer irradiation times resulted in increasing yields of the methyl ether. However, unlike **1**, the formation of secondary photoproducts (<3%—not identified) was also observed at longer irradiation times.

The quantum yield for photomethanolysis Φ_p of **9** from irradiation of **1** in 1:1 CH_3OH/H_2O (pH 7) was determined to be 0.18 ± 0.02 by comparison to the known absolute Φ_p of *o*-hydroxybenzhydrol in 1:1 CH_3OH/H_2O ($\Phi_p = 0.46$ [15]). The quantum yield of **11** from **7** was measured to be 0.21 ± 0.02 relative to the value obtained for **9**. Interestingly, Φ_p for irradiation of **7** is less than half that obtained for irradiation of *o*-hydroxybenzhydrol. This may be attributed to the electron-withdrawing nature of the pyridine nitrogen reducing the electron-donating effect of the excited state phenolate which has been shown to be required for quinone methide formation in similar systems [13–18].

3.2.2. Nuclear overhauser effect (NOE) experiments

NOE spectra were required for definitive assignment of the methyl ether product from **1** to **9**, rather than **10**, due to the close proximity of the methylene signals (δ 4.5 and 4.53 ppm). Irradiation of the pyridine ring proton



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(δ 7.93 ppm) resulted in growth of the peak at δ 4.5 ppm, allowing the assignment of these protons to the methylene group *meta* to the phenol (Fig. 1a). Irradiation of the δ 4.5 ppm methylene signal yielded a growth at δ 7.93 ppm with only a small growth in the signal from the methoxy protons (δ 4.53 ppm) (Fig. 1b). In contrast, irradiation of the methylene signal at δ 7.93 ppm gave a significant growth in the signal from the methoxy protons (Fig. 1c). This implies that the protons appearing at δ 4.53 ppm are spatially very close to the methoxy protons, while the those associated with the peak at δ 4.50 ppm are further removed from the methoxy group. These results are consistent with **9** rather than **10**.

3.2.3. pH studies in aqueous methanol

Photolysis of **1** was also carried out in 1:1 CH_3OH/H_2O solutions varying the pH of the water portion from 1 to 12. Three distinct regions are noted in the plot of yield versus pH (Fig. 2).

The increase in product yield above pH 9 observed for 5 min. irradiation coincides with the expected ground state pK_a of the phenolate (9–12). This is consistent with increased reactivity of the phenolate in the excited state, compared to the phenol, as was previously noted for the formation of quinone methides from hydroxybenzyl alcohols [13–18]. Although thermal formation of **4** has been reported [21] from **6** at physiological temperature, no reaction was observed from alkaline solutions of **1** at room temperature that were not irradiated. This lack of reactivity can be attributed to the poor leaving-group ability of hydroxide compared to the ammonium ion. The increase in product formation between pH 1 and 3 likely coincides with protonation of the benzylic alcohol in the excited state, resulting in H_2O as a leaving group, rather than hydroxide.

3.2.4. Aqueous acetonitrile with EVE

In an attempt to trap the suspected quinone methide (**5**), **1** was photolyzed in the presence of ethyl vinyl ether (EVE 1 ml) for 15 min in 1:1 CH_3CN/H_2O at pH 7. A racemic mixture of regioselective chroman products **12** was isolated in high yield following work-up, as evidenced by the ^{1}H NMR (Fig. 3). Formation of these products is consistent with an inverse demand [4 + 2] Diels–Alder type reaction of a quinone methide with a dienophile. Irradiation at low

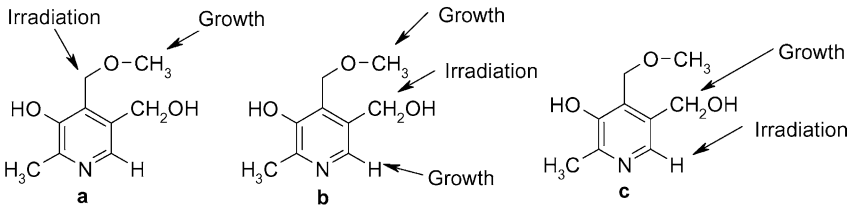


Fig. 1. NOE difference experiments on the photomethanolysis product from **1** irradiation: (a) δ 4.5 ppm; (b) δ 4.53 ppm; (c) δ 7.93 ppm.

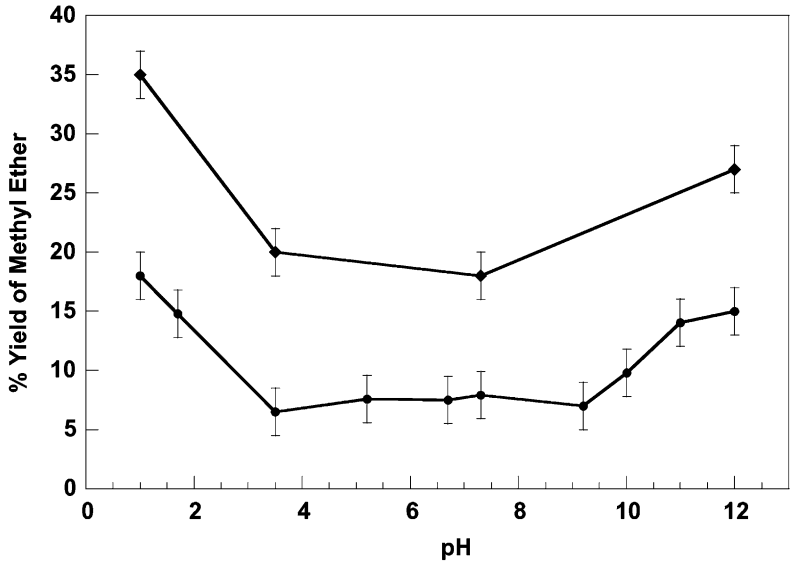


Fig. 2. Relative yields for formation of **9** upon irradiation of **1** at various pH values (54 mg in 80 ml 1:1 CH_3OH/H_2O, 254 nm, eight lamps): 5 min irradiation (●); 15 min irradiation (◆).

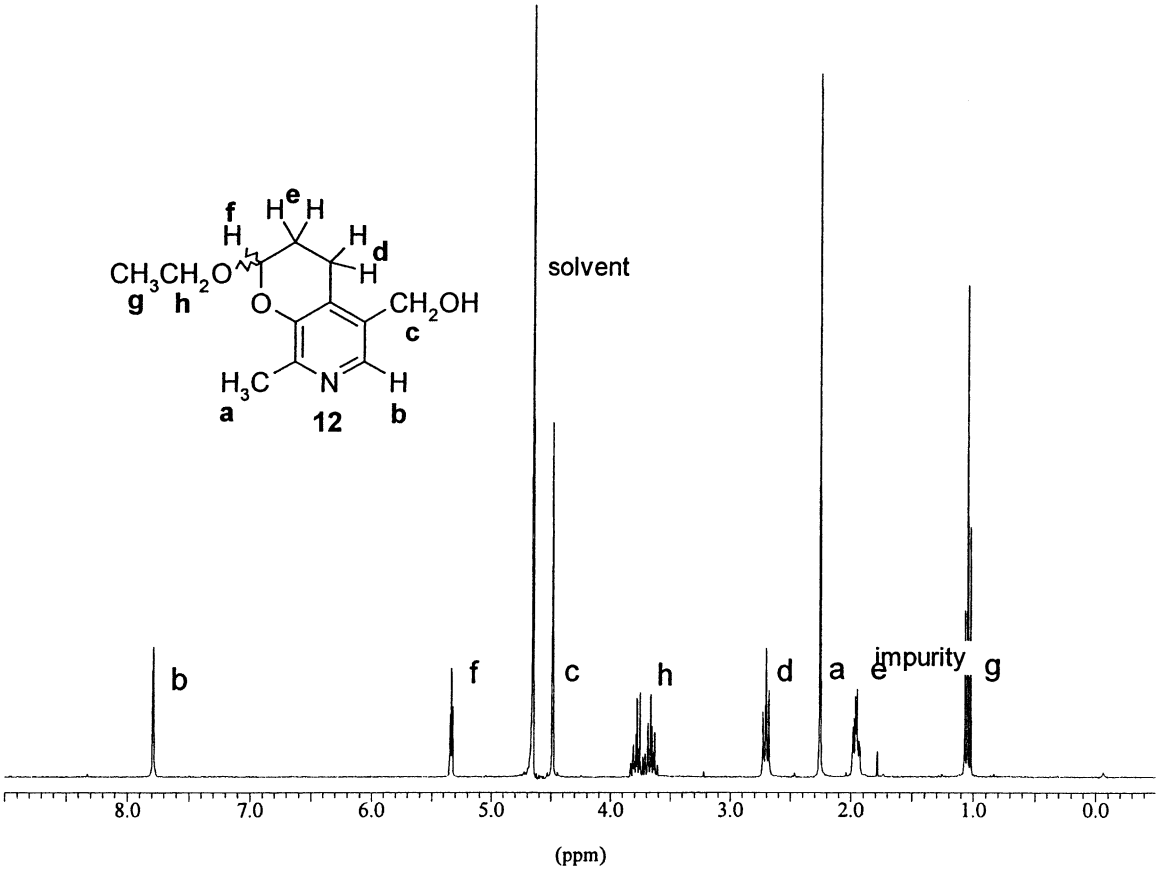
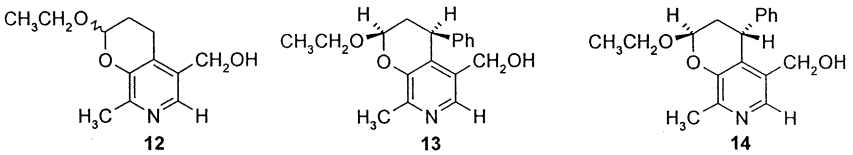


Fig. 3. ^{1}H NMR (D_2O) spectrum of **12**.

and high pH gave little or no yield of **12**, presumably due to thermal reaction of EVE in the acidic and basic media.

Exhaustive photolysis of **7** in the presence of EVE in 1:1 CH_3CN/H_2O gave a regioselective mixture of diastereomeric chroman products (**13** and **14** and their respective enantiomers). ^{1}H NMR of the isolated products (Prep TLC, 100% ethyl acetate) suggests that the diastereomers are present in a 3:2 mixture, although COESY and NOESY experiments were unsuccessful in determining which of **13** or **14** is present in the greatest amount.

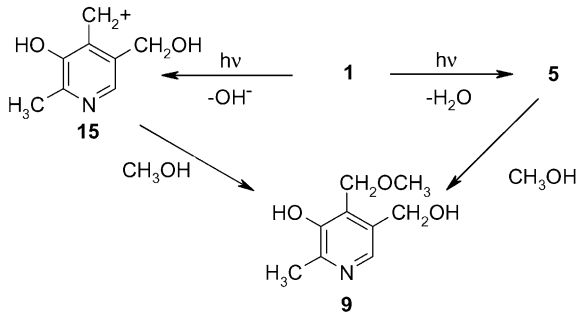


3.2.5. Implications regarding intermediates and mechanism

Formation of the respective methanolysis products (**9** and **11**) upon irradiation of **1** and **7** in CH_3OH/H_2O solutions is consistent with nucleophilic attack on either a carbocation (e.g. **15**), or a quinone methide-type intermediate (e.g. **5**) (Scheme 1, shown for **1**). Photodehydroxylation to yield carbocations is a well-known process for di- and triaryl alcohols [25–33]. However, the fact that chroman-like products (e.g. **12**) were isolated from irradiation with EVE favors the quinone methide pathway in neutral solution, as such products require the intermediacy of a 1,4-dipolar intermediate such as **5**. Photodehydroxylation in alkaline solution necessarily results in the *o*-quinone methide-type intermediate upon excitation, rather than the cation, as the phenol is already deprotonated prior to excitation. These arguments rule out the possibility of a carbocation intermediate being responsible for the observed chemistry at high and neutral pH. At low pH photodehydroxylation can be catalyzed by acid, and carbocation formation likely occurs upon excitation of the molecules under study. Quinone methide formation is also a possibility, as the excited state phenol can deprotonate in even acidic media [34].

3.3. Laser flash photolysis (LFP)

Previous work on the hydroxybenzyl alcohols has indicated that the respective quinone methides are formed



Scheme 1.

in high yield and exhibit strong signals in LFP experiments [15,16,18,19]. In order to provide further evidence of quinone methide formation upon photolysis of **1**, LFP studies were carried out with either 266 or 308 nm excitation. Experiments were conducted on **7** and **8**, rather than **1**, as the α-aryl substituents are thought to increase the conjugation and stability of the corresponding photogenerated quinone methides.

3.3.1. Transient generation and pH effect

LFP of **7** in pH 12 aqueous solution (in the phenolate form) gave two strong bands at 370 and 430 nm (Fig. 4), each consisting of a long-lived single exponential decay ($\tau \sim$ 2 ms) to an even longer lived residual decay (<10%). Due to the similarities in lifetime, these two bands are believed to arise from the same transient species. LFP of **7** in pH 7 aqueous solution also yielded peaks at 370 and 430 nm, however, the relative intensities were reversed and both signals were weaker compared to the work in alkaline solution. Each of these bands was found to consist of two single exponential decays ($\tau \sim$ 200 and ∼10 ms), indicating that two species with overlapping absorption spectra are formed upon excitation of **7** under these conditions. The true extent of the 370 nm band in both solutions is masked due to bleaching below 350 nm, caused by absorption of ground state **7**. Upon irradiation in acidic solution (pH 1), the lower band shifts below 350 nm ($\tau \approx 4$ and 50 μs) and the 430 nm band disappears.

Transient absorption spectra, similar to those obtained from **7**, were also observed from LFP studies of **8** at pH 1, 7 and 13. The lifetimes of the respective transients were qualitatively the same as those obtained for **7** at pH 1 and 7. In the case of the pH 13 runs, however, the observed lifetime of the transient (370 and 430 nm) was reduced to ∼200 μs, presumably as a result of increased quenching by the higher concentration of hydroxide (0.1 M). These results are summarized in Table 1. At all pH values the transients from both **7** and **8** exhibit the same respective ΔA_{max} and lifetimes upon photolysis in N_2 or O_2 purged solutions, implying that formation of these intermediates likely occurs through the singlet manifold.

3.3.2. Quenching studies

The fact that these transients are observed in 100% H_2O (55 M), which can itself react as a nucleophile, is indicative of their relative stability. The lifetime of the transient is only reduced by a factor of ∼5 in pH 12 solutions (0.01 M NaOH), even though hydroxide is a much better nucleophile than both methanol (∼1×10^5 times more reactive) or bromide (∼10 times more reactive). Hence, it is not surprising that experiments on **7** and **8** in neutral solution (pH 7) showed no quenching effect on either band (370 or 430 nm) with the addition of up to 3 M CH_3OH or up to 0.5 M NaBr.

Quenching studies using ethanolamine were conducted in aqueous solutions at pH 12.75 where changes in pH due to

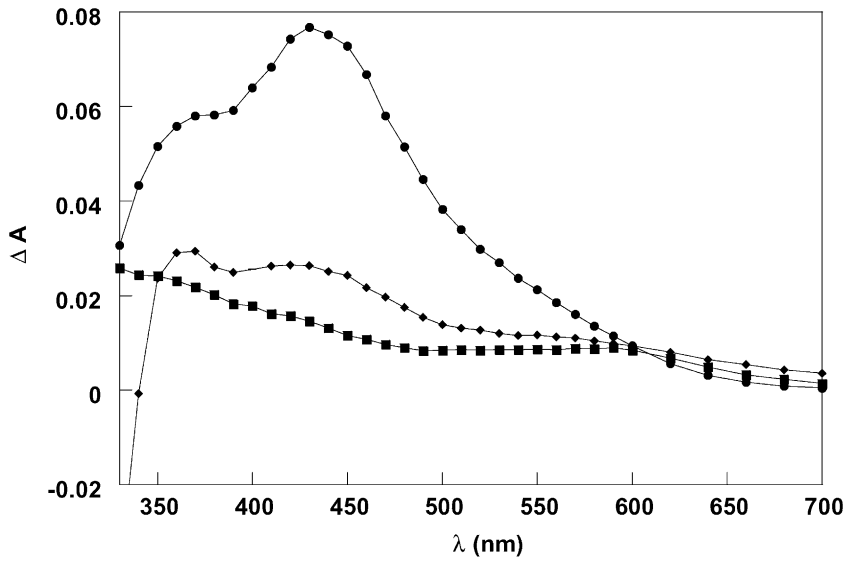


Fig. 4. Transient absorption spectra observed for **7** (λ_{ex} = 308 nm excitation, 100% H_2O, purged with O_2, 1.2 μs after excitation): pH 1 (■); pH 7 (◆); pH 12 (●).

Table 1
The pH effects on the absorption maxima and lifetimes for transients observed in LFP experiments for **7** and **8** in 100% H_2O

pH	**7** (λ_{max}, τ)	**8** (λ_{max}, τ)
1 (0.1 M HCl)	350, ~4 μs and <50 μs	350, ~4 μs and <50 μs
7	370 and 430 nm, ~200 μs and >10 ms	370 and 430 nm, ~150 μs and >10 ms
12 (0.01 M NaOH)	370 and 430 nm ~2 ms	–
13 (0.1 M NaOH)	–	370 and 430 nm, ~200 μs

the addition of the quencher were negligible and the phenolate was the only form of **7** present (as evidenced by UV–VIS spectra). Significant quenching was observed in the absorption bands at both 370 and 430 nm upon addition of small amounts of ethanolamine (0.13 M) (Fig. 5). A plot of k_{obs} versus [ethanolamine] (Fig. 5, inset) gave a straight line at low concentration of the quencher ($k_q \sim 5 \times 10^4\ M^{-1}\ s^{-1}$ for both the 370 and 430 nm bands), providing evidence that the transients observed at these two wavelengths are the same species. The long-lived residual transient observed at both wavelengths is still present, even in solutions of 2.7 M ethanolamine. Similarly, the fast decay observed upon addition of ethanolamine ($\tau \sim 2$ μs) (Fig. 5) is constant at all quencher concentrations. This indicates that these species are not electrophilic in nature and cannot be quinone methides.

3.3.3. Assignment of transients

The lack of oxygen quenching on the transients in LFP, as well as the observed reaction with nucleophiles in the quenching studies, suggests that the transient signals observed upon photolysis of **7** and **8** arise from either a carbocation (e.g. **16**) or a quinone methide-type intermediate (e.g. **17**). Although the carbocation pathway has been ruled out as a possible intermediate leading to products in the photolysis studies, this does not preclude its observation as a transient in LFP studies. The intermediacy of such a species can, however, be discounted based upon the lifetimes of the observed transients. Extensive studies by several groups have shown that it is possible to generate di- and triarylmethyl carbocations upon photolysis of fluorenols [25–30], xanthenols [31–33] and related systems [35,36]. The lifetimes of the diaryl cations (1:4 CH_3CN/H_2O—without added nucleophiles) are typically in the pico- to nanosecond range, while the triaryl systems can live longer. In the pyridoxine systems the electron withdrawing nature of the pyridine nitrogen would be expected to yield a shorter lifetime for the cation than that observed for similar diaryl systems. However, the transients generated upon photolysis of **7** and **8** are quite long lived, being in the millisecond range at pH 7 and 12 and the microsecond range at pH 1, suggesting an enhanced stability of the intermediates over that of a simple cation. Based upon these lifetime arguments, the possibility of the transient signals from **7** and **8** arising from a carbocation is ruled out.

The single transient species observed at 370 and 430 nm in alkaline solution has been assigned to quinone methide-type intermediate **17**, generated from excitation of the phenolate form of **7**. This assignment is based upon the long lifetime of the transient and its similarity to the observed

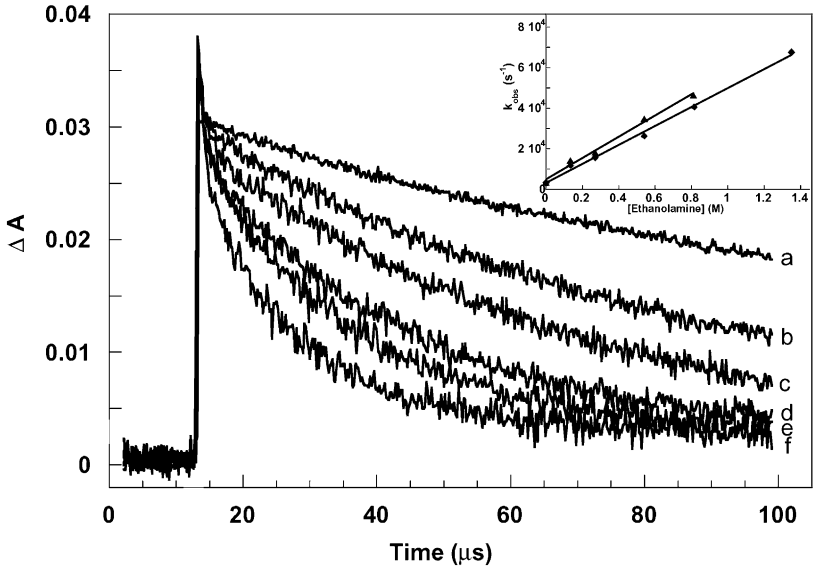
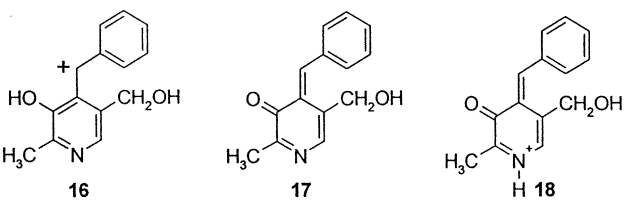


Fig. 5. Quenching of the 430 nm transient from **7** in pH 12.75 aqueous solution (normalized ΔA_{max}): (a) 0.13 M; (b) 0.27 M; (c) 0.54 M; (d) 0.81 M; (e) 1.35 M; (f) 2.7 M ethanolamine. Inset: plot of k_{obs} vs. [ethanolamine]: 370 nm (▲); 430 nm (◆).

absorption spectrum of the *o*-quinone methide (λ_{max} = 450 nm, $\tau \sim 10$ s) derived from *o*-hydroxybenzhydrol upon irradiation [15,16], as well as the observed lifetime quenching of this intermediate by an added nucleophile (ethanolamine) in alkaline solution.

Based upon the observation of two mono-exponential decays in neutral solution at both 370 and 430 nm ($\tau \sim 200$ μs and >10 ms) it appears that two quinone methide-type transients are overlapped in this region. These must arise from excitation of the neutral form of **7**, as the phenolate does not exist at pH 7. The longer of these lifetimes is consistent with the formation of **17** in neutral solution, where it is expected to react more slowly than in alkaline solution ($\tau \sim 2$ ms versus >10 ms). The decreased transient yield at neutral pH, compared to that at pH 12 (ODs matched at λ_{ex}) provides further evidence for the existence of **17**. Quinone methide formation from the similar hydroxybenzyl alcohols and hydroxybenzhydrols has been shown to increase significantly at high pH, due to electron donation from the excited state phenolate aiding in dehydroxylation [13–18].

Although the long lived species observed at 370 and 430 nm in neutral solution can be assigned to **17**, it cannot be entirely responsible for observed reactivity at this pH. The parent compound 1 has been shown to exist mainly as the zwitterionic form (**1b**) in neutral solution [5], and it seems reasonable that this is also the case for **7**. Excitation of the zwitterionic form of **7** is not expected to give **17**, as the pyridine nitrogen becomes more basic in the excited state (e.g. protonated quinolines [34]) and hence, does not lose the proton upon excitation. Although **17** cannot be formed from the zwitterion, the product studies of **1** and **7** with EVE provide strong evidence for the existence of a quinone methide-type intermediate at neutral pH, through the formation of the respective chroman systems. The only such intermediate that can be formed upon irradiation of the zwitterion is **18**, the protonated form of **17**. The lifetime of **18**, would be expected to be reasonably short, even in neutral pH, due to the large electron-withdrawing ability of the protonated pyridine nitrogen. As such, the short lived transient observed at 370 and 430 nm is tentatively assigned to this intermediate. Unfortunately, the lack of observed quenching by methanol or NaBr of the long or short lived species precludes a more definitive assignment at this pH.



Further support for the existence of **18** comes from the UV–VIS studies on these systems which have shown that the protonated form of pyridoxine (**1a**) is blue shifted significantly from the neutral form **1b**. Thus, **18** would be expected to have an absorption maximum 30–40 nm further in the blue region of the spectrum than **17**. Due to absorption by ground state **7**, it is impossible to accurately determine the existence of any absorption bands below 350 nm. However, the increase in the intensity of the 370 nm band relative to the 430 nm band between irradiation of **7** at pH 12 and 7, respectively, implicates that the absorption maximum of **18** is blue shifted relative to **17**.

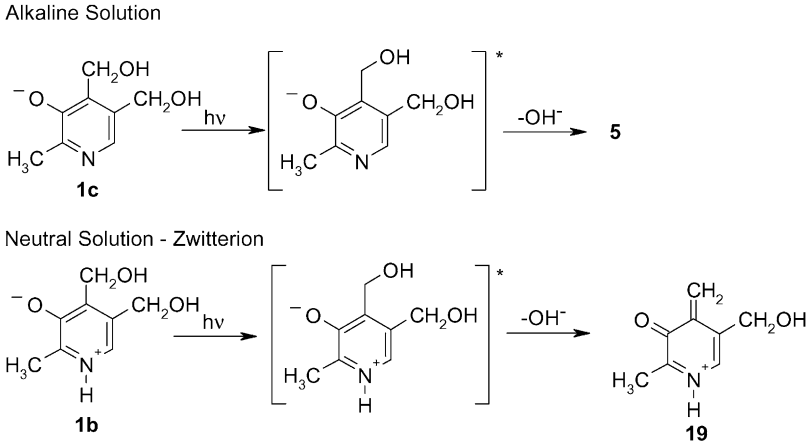
Formation of the *o*-quinone methide-type intermediate **18** is also a possibility at low pH, based upon the ability of the excited state phenol to deprotonate even in acidic media [34]. In this case, the fully protonated form of **7** would be the species being excited. However, not enough evidence has been acquired to differentiate between the suspected quinone methide and the cation at this pH. Assuming that **18** is formed in acidic solution upon irradiation of **7**, the reduced lifetime observed in LFP can be attributed to the strong electron-withdrawing effect of the protonated nitrogen making the intermediate more reactive.

3.4. Mechanism of reaction

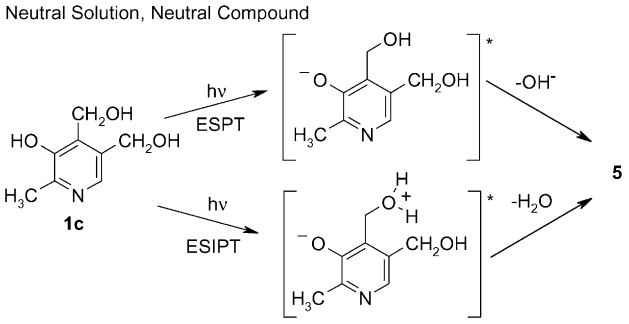
From the product studies and LFP data, it is apparent that photolysis in aqueous solution at neutral and high pH results in the formation of at least one quinone methide-type intermediate via the singlet manifold from each of **1**, **7** and **8**. The formation of these intermediates necessarily requires the formal loss of water in the excited state. Two possible pathways are available for this to occur: excited state proton transfer (ESPT) to solvent, with loss of hydroxide, and excited state intramolecular proton transfer (ESIPT) from the phenol to the benzylic alcohol, with subsequent or concerted loss of water.

In alkaline solution, the distinction between these two pathways is irrelevant, as there is no proton available for transfer to either solvent or the alcohol from the phenolate. In this case, it can be assumed that dehydroxylation to give the quinone methide-type intermediate is aided by the electron-donating ability of the excited state phenolate (Scheme 2, shown for **1**), as has been suggested for irradiation of the hydroxybenzyl alcohols and hydroxybenzhydrols in alkaline solution [13–18]. As pyridoxine (**1**) has been shown to exist mainly as the zwitterion (**1b**, Eq. (1)) at pH 7, it is likely that reaction of **1**, **7** and **8** in neutral solution to yield the suspected protonated quinone methides (e.g. **18** or **19**, the equivalent from **1**) also occurs via this pathway (Scheme 2).

ESPT to solvent from the neutral molecule is implied by the work at high pH through the increased yield upon irradiation of the phenolate (Scheme 3). However, this does not rule out ESIPT between the phenol and the benzylic alcohol, due to the close proximity of the two functional groups. (Scheme 3, shown for **1**). Wan and Chak [12] have proposed that ESIPT between these functionalities is actually responsible for the increased reactivity of *o*-hydroxybenzyl alcohol compared to respective *m*- and *p*-systems, where such a process cannot occur.



Scheme 2.



Scheme 3.

Such an intramolecular process may also explain the observed lack of photomethanolysis products from the *m*-quinone methide. Previous work has shown the quantum yield of *m*-quinone methide formation is significant relative to that of the respective *o*-quinone methide (e.g. $\Phi_p = 0.46$ [15] from *o*-hydroxybenzhydrol and 0.40 from *m*-hydroxybenzhydrol) [20]. Although the correct functionalities are present for formation of both species from **1**, it appears that the *o*-quinone methide is the only one formed. The simplest explanation is that the phenolate is better able to donate electrons to the position *ortho* to itself than to the position *meta* (as it can form a Kekulé resonance structure). Alternatively, a hydrogen-bonding interaction between the phenol and the *o*-alcohol could preclude *m*-quinone methide formation through ESIPT to yield the *o*-quinone methide, at least in cases where the proton is available for transfer.

4. Summary

Through product and LFP studies, the formation of reactive quinone methide-type intermediates has been shown to occur upon irradiation of Vitamin B_6 and some derivatives in aqueous solution at pH 7. While formation of a neutral quinone methide-type intermediate has been shown in neutral and alkaline aqueous solution, we believe that a second such intermediate, protonated at the pyridine nitrogen, also exists.

Quinone methides have been shown to be potent cancer causing agents in biological systems, due to their extremely electrophilic nature, and their ability to alkylate DNA and other biomolecules. The formation of such a reactive intermediate from **1** under essentially physiological conditions may explain some of the toxicological effects linked to the intake of large doses of this biologically relevant molecule. Previous studies [3,4] have suggested in vivo formation of a quinone methide-type intermediate from **1** can occur thermally, via interaction with an enzyme. This work provides evidence of an alternative route to the same quinone methide, requiring only light to proceed.

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